THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	David Litchfield
DEPARTMENT	Biochemistry
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PHONE NUMBER	519-661-2111 ext 86849 (lab) ext 84186 (office)
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EMAIL	litchfi@uwo.ca

Location of experimental work to be carried out: Building(s) _____MSB_____ Room(s) 355/359/365A/380

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: Canadian Cancer Society Research Institute, Canadian Institutes of Health Research, Cancer Research Society, Inc
GRANT TITLE(S): Regulation and role of protein kinase CK2 in cell cycle progression (Canadian Cancer Society Research Institute), Control of cell proliferation & survival: Convergence of CK2 & caspase signaling pathways (Canadian Institutes of Health Research), Rational Design of Novel Inhibitors of Cancer Cell Proliferation (Cancer Research Society, Inc)

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Laszlo Gyenis	lgyenis@uwo.ca	2007
Michelle Gabriel	mgabrie5@uwo.ca	2009
Dana Onica	donica2@uwo.ca	2010
Brendan Innes	binnes22@uwo.ca	2008
Stephanie Zukowski	szukowsk@uwo.ca	2010
Jacob Turowec	jturowec@uwo.ca	2006
Greg Vilk	gvilk2@gmail.com	2007

Giovanni Poggenpoel	gpoggenp@uwo.ca	2010
Miranda Hunter	mhunte47@uwo.ca	2010
~~Kathryn Garside~~	~~kgarside@uwo.ca~~	~~2009~~
~~Samantha Pillon~~	~~spillon3@uwo.ca~~	~~2010~~

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Use: All biological agents and/or biohazardous substances are used according to the UWO Biosafety Guidelines and Procedures Manual following good microbiological laboratory practices. All trained laboratory personnel are working with biological agents and/or biohazardous substances only inside the containment Level 2 laboratories. The cell lines are handled in the biological safety cabinets that are re-certified annually. Storage: All biological agents and/or biohazardous substances are stored only inside the containment laboratory that has lockable doors. The containment laboratory is always kept locked when it is unoccupied. **Disposal:** All biological agents and/or biohazardous substances are autoclaved, incinerated or disinfected to inactivate the biological agents prior to disposal. Autoclaving of biological agents are done on-site in functioning autoclaves.

Please include a one page research summary or teaching protocol.

Regulation and role of protein kinase CK2 in cell cycle progression (Canadian Cancer Society Research Institute). The growth and division of cells is controlled by a network of enzymes known as protein kinases that frequently contribute to tumorigenesis when they become active at the wrong time in cancer cells. Our research is focused on one group of these protein kinases known as CK2 because this group of enzymes is present at abnormally high levels in many forms of human cancer and because CK2 has been shown to promote breast cancer and leukemia in mice. We are working to understand how CK2 contributes to the inappropriate division of cancer cells with the hope that this information may provide new ideas for improving cancer treatment. To complement this work, we have been involved in the evaluation of novel CK2 inhibitors to determine whether these compounds can inhibit CK2 functions in cells and to examine whether they have any other undesirable effects on the cells. The latter studies are essential if CK2-targeted therapies are to move forward for clinical testing. One aspect of this project is focused on performing a detailed characterization of new compounds that have been designed, on the basis of the three-dimensional structure of CK2, to inhibit CK2 in cells. We will employ new methods, involving proteomics and genetic approaches, to characterize the effects of these compounds on cancer cells to ensure that these compounds do indeed target CK2 in cells and to determine whether these compounds have other cellular targets or side effects. A second aspect of this project will be focused on determining how CK2 is involved in the control of cell division since this function of CK2 is likely to be related to the ability of CK2 to promote tumorigenesis. This aspect of the work will be fostered by an examination of unique experimental systems (human cancer cells that have been engineered to express mutant forms of CK2) that we have developed.

Control of cell proliferation & survival: Convergence of CK2 & caspase signaling pathways (Canadian Institutes of Health Research). The overall objective of our ongoing studies is directed towards elucidation of the molecular mechanisms that control cell proliferation and survival. Biochemical and genetic studies have demonstrated the existence of networks of protein kinases that are involved in the regulation of cellular events that determine whether cells live or die. Cells also contain networks of proteases, including aspartic-acid specific proteases known as caspases that play a major role in the regulation of cell survival through their involvement in the initiation and execution phases of apoptosis. Perturbations in regulatory protein kinase and caspase networks induce alterations in cell survival and frequently accompany transformation and tumorigenesis. Furthermore, the convergence of protein kinase and caspase signaling pathways has become increasingly evident, as phosphorylation of caspase substrates within caspase recognition motifs has been shown to prevent caspase-catalyzed cleavage of a number of proteins. To systematically investigate the global role of phosphorylation in the regulation of caspase signaling, we employed computational approaches to identify protein sequences from the human proteome that contained overlapping protein kinase and caspase recognition motifs. Notably, the constitutively active and oncogenic protein kinase CK2 emerged as the most prominent kinase displaying an overlapping consensus for phosphorylation of caspase recognition motifs. HYPOTHESIS: On the basis of these observations and a striking overlap between the substrate specificity of CK2 and caspases, the working hypothesis for our studies is that CK2 protects cells from apoptosis through its ability to modulate the susceptibility of regulatory cellular proteins to caspase cleavage. To test this hypothesis, we have employed systematic computational and functional proteomic approaches that revealed many novel candidate targets with overlapping CK2 and caspase sites including pro-caspase 3 and pro-caspase 8. As a logical extension of our work, the two major aims of this project will be: i) to validate candidate proteins as direct CK2 and caspase targets, and ii) to elucidate mechanisms controlling CK2 phosphorylation of apoptotic proteins. The first aim will be addressed by studies to evaluate candidates as targets for CK2 and caspase in vitro and in cells, and an examination of the functional effects of CK2 phosphorylation on apoptosis. The second aim will involve studies to determine whether CK2 activity changes in response to apoptotic stimuli and to characterize complexes between CK2 and its substrates during apoptosis. Given the emergence of CK2 as a promising candidate for molecular-targeted therapy, we envisage that our studies could yield insights that will contribute to improvements in cancer treatment in leukemia or tumors with elevated levels of CK2.

Rational Design of Novel Inhibitors of Cancer Cell Proliferation (Cancer Research Society, Inc). The long-term goal of our studies is to develop innovative knowledge-based strategies for the control of cell proliferation and those diseases such as cancer that are characterized by inappropriate cell proliferation. These studies focus on Pin1, a peptidyl-prolyl isomerase that has emerged as a potential prognostic indicator and promising therapeutic target for cancer. Based on its essential role in cell division, elevated expression in several cancers and regulatory interactions with oncogene and tumor suppressor gene product, the underlying hypothesis for our work is that Pin1 will be an effective target for the development of novel molecular-targeted cancer therapies. Towards this objective, we undertook studies employing a broad base of experimental strategies ranging from yeast genetics to structural biology that generated the most extensive mutagenic analysis of Pin1 and new insights regarding its catalytic mechanism. We also performed an unbiased screen to identify novel inhibitors of Pin1 that yielded cyclic peptides able to inhibit Pin1 at sub-micromolar concentrations. Using NMR spectroscopy, we have determined the high-resolution structure of the inhibitory peptide (CRYPEVEIC designated CCP-1) and characterized its mode of interaction with Pin1. To capitalize on our advances, the objectives of this proposal are: i) to develop specific cell-permeable inhibitors of Pin1, and ii) to perform inhibitor validation studies in cancer cells (ie. verify inhibition of Pin1 and evaluate off-target effects).

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen ? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli (DH5a)	O Yes X No	O Yes X No	O Yes X No	2		X 1 O 2 O 2+ O 3
E. coli (XL 1 Blue)	O Yes X No	O Yes X No	O Yes X No	2		X 1 O 2 O 2+ O 3
E. coli (BL21)	O Yes X No	O Yes X No	O Yes X No	2		X 1 O 2 O 2+ O 3
Saccharomyces cerevisiae	O Yes X No	O Yes X No	O Yes X No	2		X 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes X No		Not applicable

Rodent	O Yes X No		
Non-human primate	O Yes X No		
Other (specify)	O Yes X No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	X Yes O No	A431 A549 HEK293T HeLa HeLa (Tet-off) HeLa S3 Hela-Fas Jurkat LNCaP MCF7 PANC-1 Saos-2 U20S UTA6 (U2OS-derived	1 1 2 2 2 2 2 1 1 1 1 1 1 1	ATCC, Clontech, collaborative labs
Rodent	X Yes O No	3T3-L1 (LTA5) # BV2# C2C12# C57B1/6# E7 hybridoma# F9 Cells# G182# Morphs# NIH 3T3# Swiss 3T3# Note: #*for storage purposes only*	1 1 1 1 1	ATCC, Clontech, collaborative labs
Non-human primate	X Yes O No	Cos7	2	ATCC
Other (specify)	X Yes O No	BK3A (chicken) # Note: #*for storage purposes only*		collaborative labs

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES X NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or		O Yes		O 1 O 2

other Body Fluid		O Unknown		O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? X YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E. coli (DH5a, XL 1 Blue, BL21)	pBI, pRc/CMV, pTRE, pEGFP, pcDNA3.1, NME3	Invitrogen, Clontech, Addgene	**pRc/CMV constructs:** pZW6 CK2a-HA pZW16 HA-CK2a' pZW12 Myc-CK2b **pBI constructs:** pRS2 mycCK2b +HA-CK2a' pRS3 mycCK2b + CK2a-HA pcDNA3-CrmA, pcDNA3-Xiap-myc, pcDNA3-Bcl2 pET23b-Casp6-His (plasmid is for bacterial expression only)	Overexpression of gene product. Increased cell proliferation (pZW6, 16, 12; pRS2, 3). Decreased cell death (pcDNA3-CrmA, Xiap-myc, Bcl2).

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below X NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen X YES, please specify __Cos7, HEK293T O NO
- E1A oncogene X YES (HEK293T) O NO
- Known oncogenes X YES, please specify __CK2_____ O NO
- Other human or animal pathogen and or their toxins X YES, please specify __HeLa (Cells contain human papilloma virus); _HEK293T (Cells contain adenovirus)_____ O NO

4.5 Will virus be replication defective? O YES O NO X N/A

4.6 Will virus be infectious to humans or animals? O YES O NO X N/A

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES X NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? X YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_Okadaic Acid (phosphatase inhibitor)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin____ Oral - mouse - 0.15 mg/kg _____

8.4 How much of the toxin is handled at one time*? _____5ug_____

8.5 How much of the toxin is stored*? _____1000ug_____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ X NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

10.0 Plants

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____

_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

_____

_____

_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

David Litchfield

SIGNATURE

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 X 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

X YES, date of most recent biosafety inspection: ___July 22, 2010____
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _BIO-UWO-0069_____

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
The general precautions that are outlined in UWO biosafety manual section 3.0 SAFETY PRACTICES AND PROCEDURES will be followed

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

The emergency response procedures will be followed that are outlined in the UWO biosafety manual section 3.5 MEDICAL PROCEDURES AND INCIDENT REPORTING

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE: Date: June 2, 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE:_____
Date:_____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

New Info

-------- Original Message --------
Subject: Re: Fwd: Biological Agents Registry Form: Litchfield
Date: Mon, 25 Jul 2011 13:10:39 -0400
From: Laszlo Gyenis <lgyenis@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: David Litchfield <litchfi@uwo.ca>

Hi Jennifer,

The following modifications were made on our form. Please see the modified form enclosed with this E-mail:

Section 4.2 should reflect the changes in cells.

-Section 4.2 was updated. Please note the pET23b-Casp6-His plasmid is for bacterial expression only to purify Caspase6 with HIS tag and not for transfection into cells. This plasmid was added to this column as you suggested when you visited us in the lab last time. Please let me know if this plasmid has to be listed at any other place on the form.

Questions 4.4 should reflect the use of E1A oncogenes and T-antigen from HEK 293T.
-Section 4.4 was updated reflecting E1A oncogenes and T-antigen from HEK 293T.

Does the lab really culture 10 litres of *E. coli* as listed in Table 1.2?
-Our lab cultures 2L of E.coli at one time. I corrected the amounts in Table 1.2 to 2L (10L was listed on our previous permit application)

I also changed the stored okadaic acid amount in 8.5 from 100ug to 1000ug.
As recommended on the form I checked the following web sites but Okadaic Acid was not listed as regulated toxin by the United
States Select Agents Regulation:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf
http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html

Please let me know if any additional changes are needed on our form.

Best regards,

Laszlo

Laszlo Gyenis, Ph.D.
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The University of Western Ontario
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Phone: 519661-2111 ext. 86849
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-------- Original Message --------
Subject: Re: Biological Agents Registry Form (Litchfield)
Date: Thu, 02 Jun 2011 13:40:24 -0400
From: Laszlo Gyenis <lgyenis@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: David Litchfield <litchfi@uwo.ca>

Hi Jennifer,

My supervisor forwarded me your E-mail asking me to update our Biological Agents Registry Form following your suggestions.

These are the items that were changed on the form:
1. The full name of *Saccharomyces cerevisiae* was listed in section 1.2.
2. Included the genes in Table 4.2 that we transfect into cells and they are involved in cell proliferation
3. Section 4.4 was updated to reflect the use of HeLa and HEK 293 cells
4. In addition to these above I also indicated changes in laboratory personnel on the first page of the form.

Please let me know if we need to change anything else on our Biological Agents Registry Form.

Best regards,

Laszlo

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Control of Receptor-induced Signaling Complex Formation by the Kinetics of Ligand/Receptor Interaction*

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Published, JBC Papers in Press, September 4, 2002, DOI 10.1074/jbc.M207399200

Anja Krippner-Heidenreich, Fabian Tübing, Susanne Bryde, Sylvia Willi, Gudrun Zimmermann, and Peter Scheurich‡

From the Institute of Cell Biology and Immunology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany

Tumor necrosis factor (TNF) exists both as a membrane-integrated type II precursor protein and a soluble cytokine that have different bioactivities on TNFR2 (CD120b) but not on TNFR1 (CD120a). To identify the molecular basis of this disparity, we have investigated receptor chimeras comprising the cytoplasmic part of Fas (CD95) and the extracellular domains of the two TNF receptors. The membrane form of TNF, but not its soluble form, was capable of inducing apoptosis as well as activation of c-Jun N-terminal kinase and NF-κB via the TNFR2-derived chimera. In contrast, the TNFR1-Fas chimera displayed strong responsiveness to both TNF forms. This pattern of responsiveness is identical to that of wild type TNF receptors, demonstrating that the underlying mechanisms are independent of the particular type of the intracellular signaling machinery and rather are controlled upstream of the intracellular domain. We further demonstrate that the signaling strength induced by a given ligand/receptor interaction is regulated at the level of adaptor protein recruitment, as shown for FADD, caspase-8, and TRAF2. Since both incidents, strong signaling and robust adapter protein recruitment, are paralleled by a high stability of individual ligand-receptor complexes, we propose that half-lives of individual ligand-receptor complexes control signaling at the level of adaptor protein recruitment.

The majority of cell surface receptors initiate intracellular signals after ligand-mediated homo- or heteromultimerization. Members of the tumor necrosis factor (TNF)[1] ligand family typically form stable homotrimers capable of multimerizing their respective receptors (1). Essential for intracellular signal induction is the subsequent recruitment of adaptor proteins (*e.g.* members of the TNF receptor-associated factor (TRAF) family or death domain-containing proteins like TNF receptor-associated death domain protein (TRADD) or Fas-associated death domain protein (FADD)) (1). These two protein groups define the two major signaling pathways leading to either gene induction (TRAFs) or the activation of the apoptotic program via autoproteolytic cleavage of initiator caspases by induced proximity (1).

TNF binds to two membrane receptors, TNFR1 (CD120a; p55/60) and TNFR2 (CD120b; p75/80). Whereas TNFR1 seems to be constitutively expressed in most tissues, TNFR2 expression is more restricted and can be found especially in immune cells but also in endothelial and neuronal tissue (2). TNFR2 is a typical member of the non-death domain-containing subgroup of the TNF receptor family. It directly binds TRAF2, leading to the activation of NF-κB and the c-Jun N-terminal kinase (JNK). TNFR1 carries a death domain in its cytoplasmic part and therefore represents a direct activator of apoptotic caspases after recruitment of TRADD and FADD. In parallel to its cytotoxic activity (and this seems to be unique within the TNF receptor family), TNFR1 is a strong activator of gene induction. Receptor-bound TRADD serves as an assembly platform also for recruitment of TRAF2 and receptor-interacting protein (3), which act together in the activation of the inhibitor of κB kinases, leading to the activation of NF-κB (4).

Many aspects of the initial events during signal initiation of TNF, however, are poorly understood. After ligand binding, TNF receptor complexes are internalized or may be, alternatively, proteolytically cleaved (5–7). Internalization has been shown to be important for intracellular signal initiation by TNFR1 (8) but not for others like Fas (CD95, APO-1) (8, 9). Further, in some cellular systems the activation of the initiator caspase-8 was found to be necessary for Fas cluster formation (9).

Remarkably, most ligands of the TNF family are expressed as type II transmembrane proteins from which soluble ligands are formed by proteolytic cleavage. In a recent publication, we have compared the signaling capacity of the membrane-bound (memTNF) *versus* the soluble form of TNF (sTNF) and have demonstrated that TNFR2, but not TNFR1, differentially responds to these two ligand forms (10). It was shown that memTNF, when acting on TNFR2, displays a superior capacity to initiate various cellular responses in a positive cooperative manner with TNFR1. Full TNFR2 activation can even cause a shift in the phenotype of the respective cellular response to sTNF (10). In addition, we have developed a TNFR2-specific monoclonal antibody, termed 80M2, that mimics the bioactivity of memTNF when combined with sTNF. Kinetic studies with iodinated TNF revealed that 80M2 stabilizes ligand binding in terms of a prolonged receptor complex half-life (10). These data raised the hypothesis that the kinetics of receptor ligand complex formation and disintegration might at least in part determine intracellular signaling strength. Accordingly, transient binding of sTNF to TNFR2 would only allow formation of short

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[1] The abbreviations used are: TNF, tumor necrosis factor; TNFR, TNF receptor; EGFP, enhanced green fluorescent protein; FADD, Fas-associating protein with death domain; JNK, c-Jun amino-terminal kinase; mAb, monoclonal antibody; MF, mouse fibroblast; RISC, receptor-induced signaling complex; TRAF, TNF receptor-associated factor; z-VAD-fmk, benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone; TRADD, TNF receptor-associated death domain protein; memTNF, membrane-bound TNF; sTNF, soluble TNF; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline; MOPS, 4-morpholinepropanesulfonic acid.

living complexes that may inefficiently induce intracellular signals (10). On the other hand, the bioactivity of 80M2 is dependent on its dimeric IgG1 structure, since Fab fragments derived thereof are inactive (data not shown). These data raised the possibility that secondary clustering of TNF·TNFR2 complexes by 80M2 might reflect the underlying mechanism by which memTNF gains superior signaling capability on TNFR2.

Differences in the bioactivity of soluble and membrane-bound forms of other members of the TNF ligand family have also been described (*e.g.* for Fas ligand (FasL) (11), CD40L (12), and TRAIL (13)). Soluble FasL can even exert antiapoptotic activity by serving as an antagonist for the membrane-bound form of FasL (11).

In this study, we have analyzed receptor chimeras derived from the extracellular domains of the two TNF receptors and the intracellular domain of Fas. TNFR2-Fas chimera, comprising the extracellular domain of TNFR2 and the cytoplasmic part of Fas, strongly induced apoptosis only after treatment with memTNF-like stimuli but not with sTNF. In contrast, both ligand forms induced a strong apoptotic signal in TNFR1-Fas chimeras. These data show that the individual responsiveness of TNFR1 and TNFR2 for soluble or membrane-bound TNF can be transferred to a distinct intracellular signaling system, indicating that responsiveness is dominantly controlled by the extracellular domains.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—HeLa cells stably transfected with human TNFR2 (HeLa80) or TNFR2 plus Fas (HeLa80Fas) (14, 15) and Chinese hamster ovary (CHO) cells expressing TNFΔ1–12 ($CHO_{TNF\Delta1-12}$) (10) have been described elsewhere. Simian virus 40 large T-immortalized murine fibroblasts have been generated from TNFR1 and TNFR2 double knockout mice and were generously supplied by Daniela Männel (University of Regensburg, Regensburg, Germany). HeLa cells, CHO cells, and immortalized mouse fibroblasts were grown in RPMI 1640 medium supplemented with 5% (v/v) heat-inactivated fetal calf serum and 2 mM L-glutamine. 1 μg/ml puromycin A was added once a week to TNFR1-Fas and TNFR2-Fas expressing mouse fibroblasts routinely. Kym-1 cells were grown in Clicks-RPMI medium containing 10% fetal calf serum and 2 mM L-glutamine (10). Recombinant human TNF (2×10^7 units/mg) was provided by Knoll AG (Ludwigshafen, Germany). Cys-TNF and mutants derived thereof (Cys-TNF143N/145R and Cys-TNF32W/86T) have been generated in *Escherichia coli* and purified to homogeneity using a nickel-chelate column.[2] The monoclonal mouse antibody 80M2 (16), H398 (17), and Htr9 (18) have been described. The TNFR2-specific monoclonal antibody MR2–1 was kindly provided by W. Buurman (University of Limburg, Maastricht, The Netherlands). Additional antibodies specific for TNFR2 were purchased from R&D (goat anti-huTNFR2), TRAF2-specific antibodies (mouse anti-huTRAF2, clone C90–481) were from Pharmingen, and antibodies specific for JNK (rabbit anti-huJNK; C-17) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). z-VAD-fmk was purchased from Bachem. The expression plasmids pFADD-EGFP and pCaspase-8-EGFP were kind gifts from Michael Lenardo (National Institutes of Health, Bethesda, MD), and pTRAF2-EGFP was from Harald Wajant (University of Stuttgart, Stuttgart, Germany), and they have been described elsewhere (19, 20).

Measurement of the Metabolic Activity by Microphysiometry—Description and operation of the Cytosensor microphysiometer (Molecular Devices Corp., Sunnyvale, CA) have appeared elsewhere (21, 22). HeLa80Fas (3×10^5 cells) were seeded into chambers (Molecular Devices). The cell capsule was placed into a flow- and temperature (37 °C)-regulated sensing chamber of the microphysiometer, and pH changes were monitored. Cells were perfused with low phosphate-buffered RPMI medium (Irvine Scientific, Irvine, CA) in a cyclic manner. The pump cycle was 120 s, comprising a flow-on period (100 μl/min for 80 s) followed by a flow-off period (40 s). The extracellular acidification rate was mathematically normalized to 100% at the data point just prior to stimulation with TNF143N/145R (150 ng/ml) (basal acidification rate). The addition of mAb 80M2 (2 μg/ml) was performed 30 min prior to treatment with TNF mutants and extended to the whole TNF stimulation time of 30 min.

Generation of Stably Transfected Cell Lines—Expression constructs encoding the fusion proteins TNFR1-Fas and TNFR2-Fas were generated by PCR cloning. A *Kpn*I site was introduced by silent mutagenesis into the coding region of pBS-TNFR1 and pBS-TNFR2 at bp 704 and 899, respectively, 3′ of the potential transmembrane region. In addition, the cytoplasmic region of Fas was amplified by PCR introducing 5′ and 3′ appropriate restriction sites for TNFR ligation (pBS TNFR1-Fas and pBS TNFR2-Fas). TNFR1-Fas and TNFR2-Fas were subcloned into the expression vector pEF PGKpuro (23), using *Bam*HI and *Eco*RV, generating pEFpuroTNFR1-Fas and pEFpuroTNFR2-Fas. All constructs generated by PCR were verified by sequencing. Immortalized mouse fibroblasts (4×10^5 cells) from TNFR1 and TNFR2 double knockout cells were transfected with pEFpuroTNFR1-Fas or pEFpuroTNFR2-Fas using LipofectAMINE Plus (Invitrogen) according to the manufacturer's recommendations. The day after, cells were selected for stably expressing cells by 1–5 μg/ml puromycin A, and 2 weeks later they were sorted for TNFR1-Fas- and TNFR2-Fas-positive cells by flow cytometry using a FACStar$^+$ (Becton Dickinson, San Jose, CA). Briefly, 5×10^5 cells were harvested and resuspended in PBA (0.025% bovine serum albumin, 0.02% NaN_3 in PBS) containing mouse 5 μg/ml anti-huTNFR1 (Htr 9) or mouse anti-TNFR2 antibodies (MR2–1). After incubation for 1 h at 4 °C, cells were washed once with PBA, resuspended in PBA containing secondary fluorescein isothiocyanate-labeled goat anti-mouse IgG plus IgM antibodies (Dianova, Germany), and incubated at least for 30 min at 4 °C. Cells were washed again and subjected to fluorescence-activated cell sorting. 10,000–30,000 positive cells were collected and grown in cell culture medium containing 1 μg/ml puromycin A. TNFR1-Fas and TNFR2-Fas expressing mouse fibroblasts (MF-R1-Fas and MF-R2-Fas cells, respectively) were stable for at least 3 weeks.

Cell Death Assays—Mouse fibroblasts (1.5×10^4 cells/well) were grown in 96-well plates overnight. Cells were then treated as indicated and cultivated overnight. The next day, cells were washed three times with PBS followed by crystal violet staining (20% methanol, 0.5% crystal violet) for 15 min. The wells were washed with H_2O and air-dried. The dye was resolved with methanol for 15 min, and optical density at 550 nm was determined with an enzyme-linked immunosorbent assay plate reader (SPECTRAmax 340PC; Molecular Devices).

For coculture experiments, CHO cells (1.5×10^5) were seeded into a 12-well plate. The next day, MF-R2-Fas cells (1.5×10^5) were given on top and cultivated at 37 °C. Cells were visualized by light microscopy, and pictures were taken 1 h after seeding of the mouse fibroblasts.

Coimmunoprecipitation and Western Blotting—HeLa80Fas cells (5×10^6 cells) were pretreated where indicated with antagonistic TNFR1-Fab fragment (H398-Fab) for 30 min followed by stimulation with sTNF (100 ng/ml) or Cys-TNF (116 ng/ml). After incubation at 37 °C for the indicated times, cells were washed with ice-cold PBS and scraped off the plate in PBS. Cells were pelleted and lysed in 300 μl of lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 30 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, protease inhibitor mix (Roche Molecular Biosciences). Proteins were extracted by vortexing three times for 40 s, and TNFR2 complexes were immunoprecipitated for 3 h at 4 °C while tumbling. Immune complexes were washed in lysis buffer and assessed by Western blotting using TRAF2-specific antibodies. Proteins were visualized by chemiluminescence (Super Signal; Pierce).

Electrophoretic Mobility Shift Assays of NF-κB Activation—5×10^5 cells/well HeLa80Fas cells or mouse fibroblasts were seeded in six-well plates and grown overnight. Where indicated, cells were pretreated with 2 μg/ml mAb 80M2 for 30 min. The cells were then stimulated for various times with the indicated reagents. Nuclear extracts were prepared as described (24), and samples were adjusted for identical protein levels. As probe, [^{32}P]ATP-end-labeled NF-κB-specific oligonucleotides (5′-AGTTGAGGGGACTTTCCCAGGC-3′) were used.

Immunocomplex JNK Assay—JNK assays were performed basically as described (25). Briefly, following stimulation, cells (5×10^6 cells) were lysed in kinase lysis buffer (20 mM Tris, pH 7.4, 5 mM $MgCl_2$, 1% Triton X-100, 150 mM NaCl, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM $NaVO_4$, and 1 mM NaF) by sonification. JNK was immunoprecipitated with 0.6 μg of JNK-specific antiserum (Santa Cruz Biotechnology) and subjected to kinase assays using GST-Jun-(5–89) (0.5 μg/assay) and 5 μCi of [^{32}P]ATP as substrate. The reactions were carried out in assay buffer (20 mM MOPS, pH 7.2, 10 mM EGTA, 2 mM $MgCl_2$, 0.1% Triton X-100, and 1 mM dithiothreitol) at 37 °C for 20 min.

Transient Transfections and Confocal Microscopy—Cells were harvested and transiently transfected with 10 μg of expression plasmids of

[2] A. B. Hammer, J. Gerspach, P. Scheurich, and K. Pfizenmaier, manuscript in preparation.

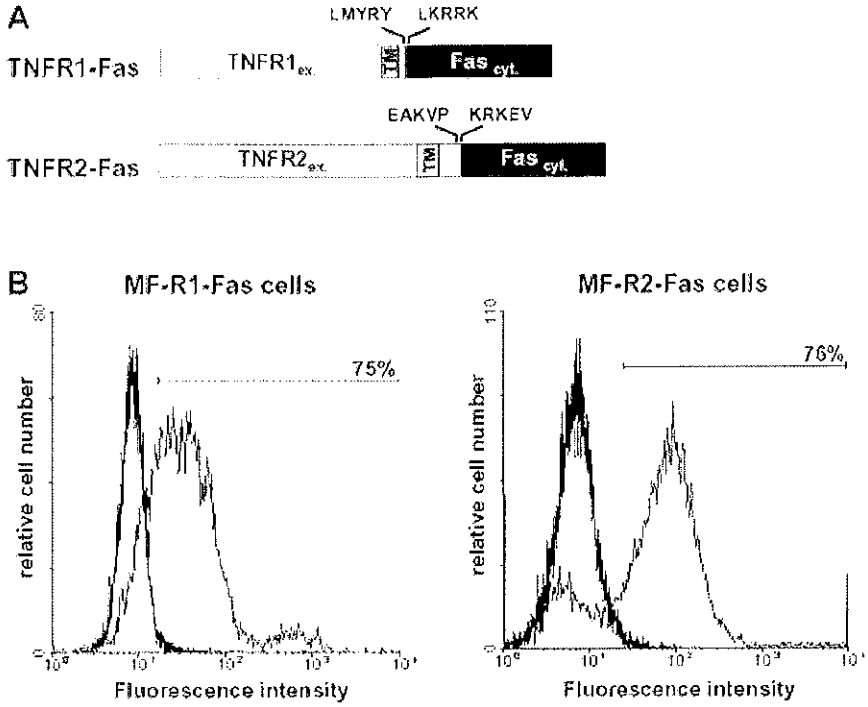

FIG. 1. **Stable expression of chimeric receptors in mouse fibroblasts.** *A*, schematic representation of TNFR1-Fas and TNFR2-Fas chimeric proteins. The cytoplasmic domain of Fas, amino acids 191–335, was fused to the C terminus of the potential transmembrane region of TNFR1 (amino acids 236) or TNFR2 (amino acids 301). Amino acids in the fused region are indicated. *B*, immortalized mouse fibroblasts were stably transfected with TNFR1-Fas or TNFR2-Fas expression plasmids. Expression of the chimeras was analyzed by flow cytometry using TNFR1-specific (Htr9) and TNFR2-specific (MR2–1) antibodies. The percentage of TNFR-Fas-positive cells is indicated.

pFADD-EGFP and pTRAF2-EGFP, respectively, or with 4 μg of pCaspase-8(C369S)-EGFP plus 4 μg of murine pFADD by electroporation. Cells (800 μl; 1.25×10^6 cells/ml) were electroporated at 1800 microfarad and 250 V in a 0.4-cm cuvette (Peqbio Easyject Plus; Peqlab). After electroporation, cells were immediately transferred into cell culture medium, and 3×10^5 cells/dish (35 mm; Maltek) were grown for 18 h before analysis. Electroporation with pCaspase-8(C369S)-EGFP required the addition of 20 μM z-VAD-fmk. For live imaging TNF, mAb 80M2 and Cys-TNF143N/145R were coupled with AlexaFluor-546 (Molecular Probes, Inc., Eugene, OR) according to the manufacturer's instructions. Cells were washed in PBS and, where indicated, preincubated with AlexaFluor-546-coupled 80M2 ($80M2_{(red)}$) (6 μg/ml) for 4 min at room temperature or 1.7 μg/ml AlexaFluor-546-coupled sTNF ($sTNF_{(red)}$) on ice followed by two washings with cell culture medium without phenol red. Cells were placed into a chamber held constantly at 37 °C and 5% CO_2 and treated where indicated with 300 ng/ml TNFR2-specific TNF mutant (Cys-TNF143N/145R), and pictures were taken at the indicated time points. Alternatively, 10^5 cells were seeded onto coverslips in a 24-well plate and treated as above. Instead of performing live imaging of the cells, cells were washed with ice-cold PBS, fixed with 3.5% paraformaldehyde in PBS for 15 min at 37 °C at various time points, mounted with Flouromount-G (Biozol, Germany) onto glass slides, and examined using a Leica DM IRBE confocal immunofluorescence microscope. Pictures were taken at a resolution of 1024 × 1024 pixels with a magnification of ×630 for live imaging and ×1000 for fixed cells.

Binding Kinetics—Receptor-ligand studies were performed as described (26). Briefly, TNF was labeled with ^{125}I by the chloramine-T method. Murine fibroblasts were incubated with 0.2 nM ^{125}I-TNF in the presence or absence of mAb 80M2 (2 μg/ml) for 1 h on ice. Cells were incubated for several time periods at 37 °C, and cell-bound ^{125}I-TNF was determined after centrifugation of the cells through a phthalate oil mixture.

RESULTS

Construction of Fibroblasts Expressing Chimeric TNF Receptor/Fas Proteins—To get a better understanding for the molecular basis of the differential signaling capacity of sTNF and memTNF, we constructed chimeras of Fas and TNFR1 and TNFR2, respectively. These fusion proteins consist of the extracellular and transmembrane region of the respective human TNFRs fused to the cytoplasmic domain of human Fas (Fig. 1*A*). Hybrid constructs TNFR1-Fas and TNFR2-Fas were stably expressed in large T antigen-immortalized fibroblasts derived from TNFR1/TNFR2 double knockout animals, obtaining a cellular system devoid of any TNF background responsiveness. Fluorescence analyses of the TNFR1-Fas- and TNFR2-Fas-expressing mouse fibroblast cells, MF-R1-Fas and MF-R2-Fas, respectively, are shown in Fig. 1*B*. Equilibrium binding studies using iodinated TNF revealed ligand binding sites of 15,000 for MF-R1-Fas cells and 45,000 for MF-R2-Fas cells per cell (data not shown).

Induction of Apoptosis in MF-R1-Fas and MF-R2-Fas Cells—As expected, we could not detect any TNF responsiveness in the parental mouse fibroblasts devoid of both mouse TNF receptors (data not shown). MF-R1-Fas cells, however, developed a strong cytotoxic response after treatment with serial dilutions of sTNF (Fig. 2*A*). Development of cell death was nearly maximum at TNF concentrations as low as 1 ng/ml, and the majority of the cells showed typical blebbing already after 3 h of sTNF treatment (data not shown). Further, cell death could be blocked by the inhibitor z-VAD-fmk, demonstrating the involvement of caspases (data not shown). Wild type TNFR1 is equally well activated by sTNF and memTNF (10). We confirmed this for TNFR1-Fas using the TNF mutein Cys-TNF32W/86T, derived from a TNFR1-specific mutant of sTNF (27), which allows additional receptor cross-linking due to the formation of cysteine-linked multimers (Fig. 2*A*). The respective TNFR2-specific mutein of TNF, Cys-TNF143N/145R, did not induce apoptosis in MF-R1-Fas cells (Fig. 2*A*).

In a parallel set of experiments, we investigated the chimeric receptor TNFR2-Fas. MF-R2-Fas cells were treated with sTNF up to concentrations of 300 ng/ml, but no significant cytotoxic response could be observed after overnight culture (Fig. 2*B*). In the presence of the antibody 80M2, however, a strong cytotoxic response was observed with a half-maximum effect at a TNF concentration of about 100 pg/ml, whereas 80M2 on its own was not toxic (Fig. 2*B*). Cell death developed rapidly; most cells showed massive signs of disintegration after 1 h of memTNF-like stimulation (data not shown). Again, induction of cytotoxicity could be efficiently blocked with the pan caspase inhibitor z-VAD-fmk (data not shown). The TNFR2-selective derivative Cys-TNF143N/145R also induced a significant apoptotic re-

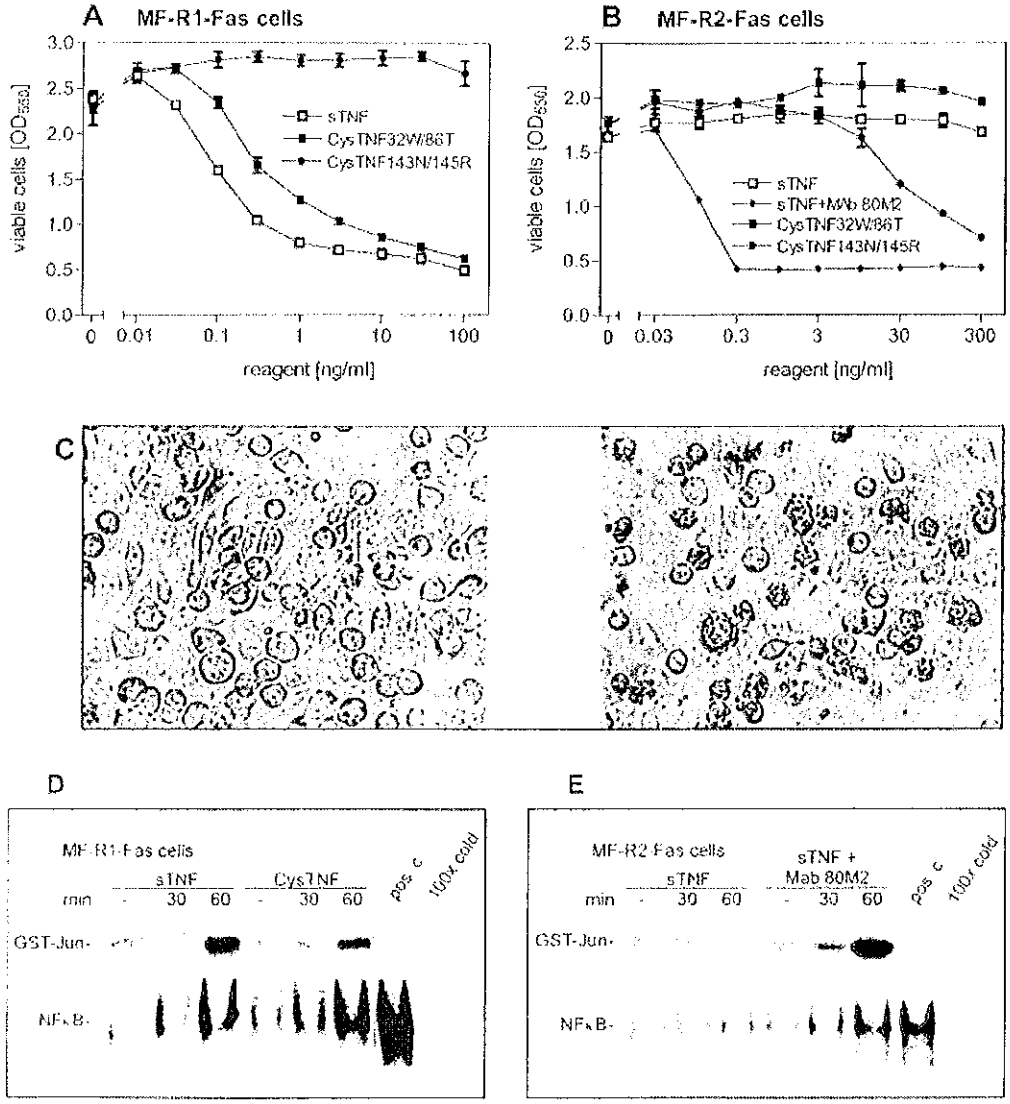

FIG. 2. **Response pattern of TNFR1-Fas and TNFR2-Fas expressing cells to sTNF and memTNF.** *A* and *B*, MF-R1-Fas (*A*) and MF-R2-Fas (*B*) cells were treated with serial dilutions of sTNF or TNFR1-specific (Cys-TNF32W/86T) and TNFR2-specific (Cys-TNF143N/145R) TNF mutants, respectively. For costimulation with the monoclonal antibody 80M2, cells had been preincubated with 2 μg/ml 80M2 for 30 min at 37 °C before sTNF treatment. Cell viability was determined by crystal violet staining the next day. All experimental groups shown were performed in parallel, one representative experiment out of three is shown. *C*, wild type Chinese hamster ovary (CHO_{wt}) cells (*left*) or CHO cells stably expressing a noncleavable form of memTNF ($CHO_{TNFΔ1-12}$) (*right*) were grown overnight. MF-R2-Fas cells were seeded on top, and induction of apoptosis was followed by light microscopy. Pictures were taken after 1 h of coculture. The percentage of apoptotic mouse fibroblasts was calculated after counting about 200 cells. *Bars*, 75 μm. *D and E*, MF-R1-Fas (*D*) and MF-R2-Fas (*E*) cells were treated with TNF (100 ng/ml), CysTNF (112 ng/ml), without or after pretreatment with mAb 80M2 (2 μg/ml) for 30 min as indicated. Cell lysates were prepared directly before (−) or after the indicated time points of TNF stimulation, and JNK activity was measured by immunocomplex kinase assay with GST-c-Jun-(5–89) as a substrate (*upper panels*), or the translocation of NFκ-B was investigated from isolated nuclei by gel shift analysis (*lower panels*). The positive control (*pos. c*) corresponds to HeLa cells stimulated with TNF (100 ng/ml) for 30 min. The specificity was controlled by the addition of a 100-fold excess of unlabeled oligonucleotides (*100× cold*).

sponse, although with reduced efficacy when compared with sTNF plus 80M2 (Fig. 2*B*). In contrast, the TNFR1-specific mutein Cys-TNF32W/86T was ineffective (Fig. 2*B*). To confirm that the transmembrane form of TNF is also able to induce apoptosis in MF-R2-Fas cells, these were cocultured for 1 h with CHO cells expressing a mutant form of memTNF that cannot be processed by the tumor necrosis factor α-converting enzyme (10). These cells (Fig. 2*C*, *right panel*), but not control CHO cells (Fig. 2*C*, *left panel*), induced a strong cytotoxic response in TNFR2-Fas-expressing mouse fibroblasts. When apoptotic cells were counted after 3 h of coculture, <5% of apoptotic MF-R2-Fas cells were determined in cocultures with control CHO cells, whereas between 68 and 79% (n = 3) of MF-R2-Fas cells showed an apoptotic phenotype in cocultures with memTNF-expressing CHO cells (data not shown). These values are in good agreement with the percentage of the MF-R2-Fas cells expressing high numbers of chimeric receptors as estimated from flow cytometry data (Fig. 1*B*). As expected, memTNF-expressing CHO cells were also toxic for MF-R1-Fas cells (data not shown). In summary, these results show that the divergent responsiveness of TNFR1 and TNFR2 to sTNF is independent of the cytoplasmic domain of the receptors, since it is transferable to the intracellular part of Fas.

Gene Induction Pathways Initiated by TNFR-Fas Chimeras—Although Fas represents the prototype of a death receptor, it is also known to activate gene expression (*e.g.* via the activation of the transcription factor NF-κB and activation of mitogen-activated protein kinases) (25, 28). To investigate

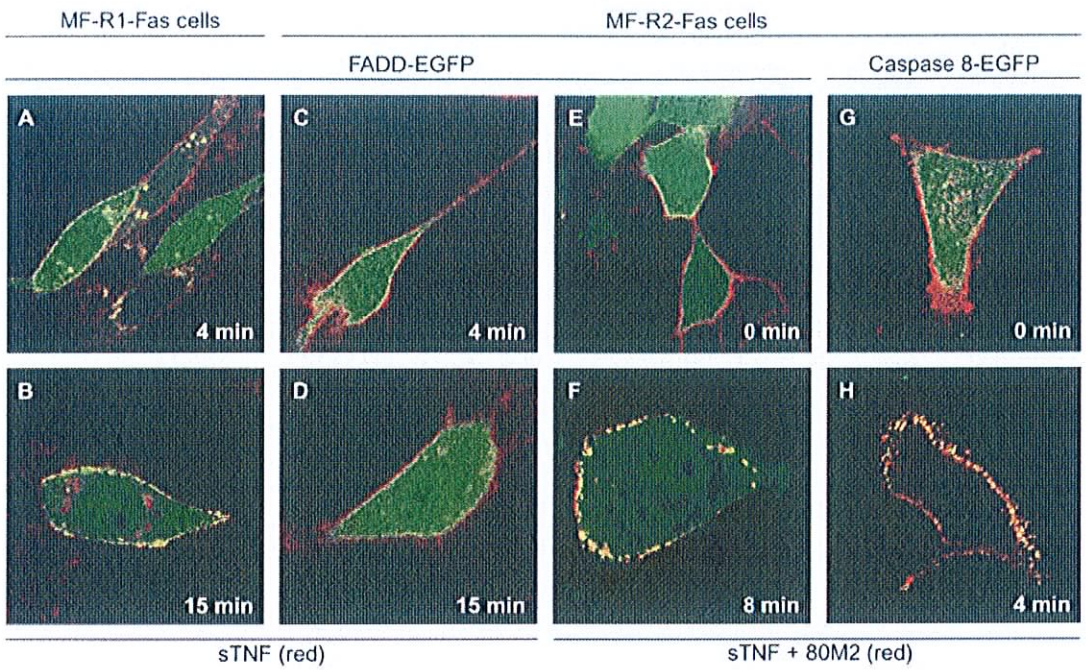

FIG. 3. **Differential RISC formation of TNFR-Fas chimeras.** MF-R1-Fas (*A* and *B*) or MF-R2-Fas (*C–H*) cells were transiently transfected with constructs expressing human FADD-EGFP (*A–F*) or human caspase-8(C360S)-EGFP plus murine FADD in the presence of 20 μM z-VAD-fmk (*G* and *H*). The day after, cells were pretreated with Alexa 546-labeled 80M2 ($80M2_{(red)}$; 6 μg/ml) (*E–H*) or sTNF ($TNF_{(red)}$; 1.7 μg/ml) (*A–D*) for 4 min on ice followed by washing with PBS. Subsequently, unlabeled sTNF (40 ng/ml) was added to the $80M2_{(red)}$-treated cells, and cells were examined by live imaging (*A–F*) or fixed at the indicated time points (*G* and *H*) and examined using a confocal laser-scanning microscope.

whether the differential responsiveness of TNFR2-Fas to sTNF and memTNF also holds true for noncytotoxic Fas responses, we analyzed the activation of NF-κB and JNK in both MF-R1-Fas and MF-R2-Fas cells after treatment with sTNF and memTNF-like stimuli. The activations of NF-κB and JNK were investigated by electromobility shift assays and immunocomplex kinase assays, respectively, revealing an identical response pattern for both cellular responses (Fig. 2, *D* and *E*) as compared with the induction of apoptosis (Fig. 2, *A* and *B*). These results strongly suggest that the molecular basis of the difference in signal initiation by memTNF and sTNF is at or upstream of the formation of the receptor-induced signaling complex (RISC). In accordance with literature data, JNK activation induced by TNFR-Fas chimera could be blocked by z-VAD-fmk (25, 29, 30), implicating the dependence on the activation of caspases, whereas nuclear translocation of NF-κB was rather augmented by this caspase inhibitor (data not shown).

Recruitment of FADD to TNF Receptor/Fas Chimeras—We then asked whether the observed differences in the signaling strength of the various ligand/receptor chimera combinations are mirrored at the level of RISC formation. One of the first intracellular reactions after ligand-induced oligomerization of Fas is the recruitment of FADD, leading to caspase-8 activation. We therefore monitored transiently expressed human FADD-EGFP in mouse fibroblast cell lines by confocal microscopy after stimulation with AlexaFluor-546-labeled TNF receptor agonists. Fig. 3 shows the overlays of the green and the red fluorescence observed. As expected, MF-R1-Fas cells transiently transfected with FADD-EGFP showed a green cytosolic fluorescence (Fig. 3A). These cells had been preincubated with Alexa-546-labeled sTNF, detectable as a weak red cell surface staining. No prominent colocalization with FADD-EGFP at the cell surface can be observed. After incubation of the cells for 15 min at 37 °C, however, the majority of the TNFR1-Fas-bound sTNF had formed clusters mostly colocalized with FADD-EGFP (Fig. 3*B*). These data demonstrate that sTNF is able to recruit significant amounts of FADD-EGFP to TNFR1-Fas molecules, leading to the formation of large RISC aggregates.

In contrast, the respective sTNF treatment of MF-R2-Fas cells did not reveal a significant colocalization of sTNF and FADD-EGFP, and no signs of cell surface located cluster formation could be observed (Fig. 3, *C* and *D*). Stimulation for only 2–8 min with a memTNF-like agent, however, consisting of sTNF and the red fluorescent antibody 80M2, induced rapid formation of cell surface-associated clusters of colocalized TNFR2-Fas and FADD-EGFP (data not shown; see Fig. 3, *E* and *F*). Similar results were obtained using MF-R2-Fas cells transiently transfected with pCaspase-8(C360S)-EGFP, expressing an catalytic inactive caspase-8, and murine FADD expression constructs in the presence of z-VAD-fmk. Again, sTNF was able to induce recruitment of caspase-8 to TNFR1-Fas but not to TNFR2-Fas chimeras (data not shown). In contrast, memTNF-like stimuli were efficient in both cellular systems (data not shown; Fig. 3, *G* and *H*). Together, these data demonstrate that the different signaling capacities of the two TNF forms are directly reflected at the level of RISC formation.

Enhanced Recruitment of TRAF2 to Wild-type TNFR2 by memTNF—We next investigated whether a lack of significant adaptor protein recruitment to TNFR2-Fas by sTNF can also be observed in wild type TNFR2-positive cells. In HeLa cells stably overexpressing TNFR2 (HeLa80), transiently expressed TRAF2-EGFP was also primarily located in the cytosol (Fig. 4, *upper panel*). The addition of the TNFR2-selective sTNF mutant sTNF143N/145R, marked with a red fluorescing dye, revealed a staining of the plasma membrane but did not result in any visible recruitment of TRAF2-EGFP to the cell membrane (Fig. 4*A*, *upper panel*). Cellular stimulation with sTNF143N/145R in the presence of the antibody 80M2, however, resulted in the strong formation of membrane-associated TRAF2-EGFP aggregates showing colocalization with TNFR2 (Fig. 4*A*, *lower panel*). Significant TRAF2-EGFP recruitment could also be induced using the secondary cross-linked TNFR2-selective TNF mutein Cys-TNF143N/154R (data not shown). Similar

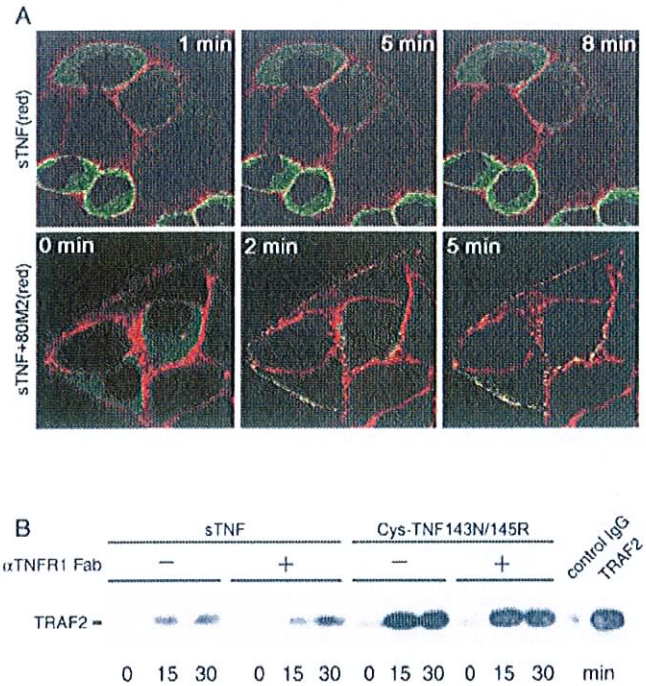

FIG. 4. **Recruitment of TRAF2 by stimulation of TNFR2 with a memTNF-like agent but not with sTNF.** *A*, HeLa cells, stably expressing TNFR2 (HeLa80), were transiently transfected with constructs expressing huTRAF2-EGFP. The day after, cells were pretreated with Alexa 546-labeled mAb 80M2 (6 μg/ml) or TNFR2-specific TNF (sTNF143N/145R; 1.7 μg/ml) for 4 min on ice followed by washing with PBS. Subsequently unlabeled membrane-like TNF (Cys-TNF143N/145R) was added to the mAb 80M2-treated cells. Live imaging was performed with a confocal microscope, and pictures were taken at the indicated times. *B*, HeLa cells stably expressing TNFR2 plus Fas (HeLa80Fas) were treated with sTNF (100 ng/ml) or Cys-TNF143N/145R (116 ng/ml) for the indicated times, followed by cell lysis. Where indicated, cells had been pretreated with antagonistic TNFR1-Fab fragments (αTNFR1-Fab; 14 μg/ml) to prevent binding of sTNF to TNFR1 and subsequent TRAF2 recruitment. Lysates were subjected to coimmunoprecipitation with TNFR2-specific antibodies, and Western blot analysis was performed using TRAF2-specific antibodies. As controls, TNFR2-specific antibodies (control IgG) and lysates from cells overexpressing huTRAF2 (TRAF2) were used.

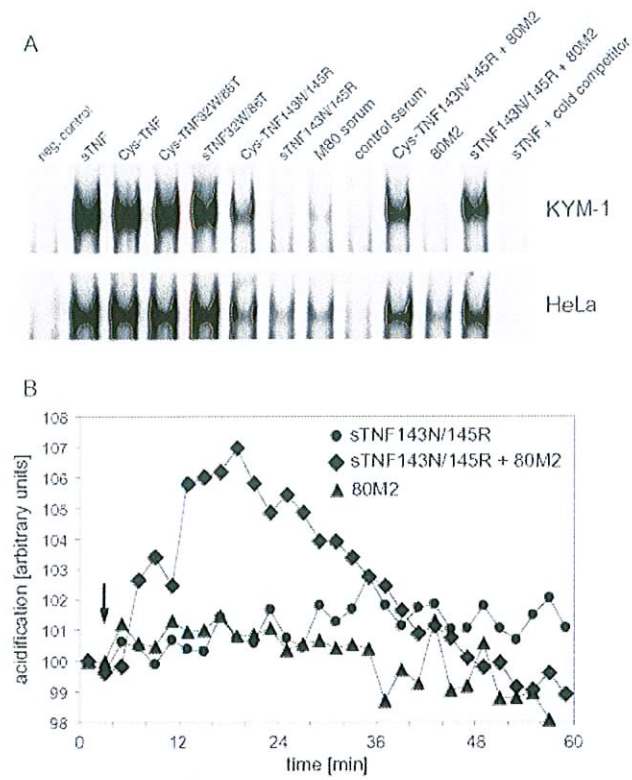

FIG. 5. **Enhanced NF-κB activation and metabolic activity induced by memTNF-like stimuli.** *A*, KYM-1 cells (*top*) and HeLa cells expressing TNFR2 (HeLa80, *bottom*) were treated with various sTNF-like stimuli (sTNF, 30 ng/ml; TNFR1-specific sTNF32W/86T, 30 ng/ml; TNFR2-specific sTNF143N/145R, 300 ng/ml) or memTNF-like stimuli (Cys-TNF, 30 ng/ml; sTNF/80M2, 30 ng/ml/2 μg/ml; TNFR1-specific Cys-TNF32W/86T, 30 ng/ml; TNFR2-specific Cys-TNF143N/145R, 300 ng/ml) for 30 min. An agonistic TNFR2-specific serum (M80, 1:200) and a rabbit control serum (1:200) were also included. Nuclear extracts were prepared, and 10 μg of protein was subjected to gel shift analysis using ^{32}P-labeled NF-κB-specific oligonucleotides. *B*, the metabolic activity of HeLa80Fas cells was analyzed using a microphysiometer. HeLa80Fas cells (3×10^5) had been pretreated with mAb 80M2 for 30 min at 37 °C where indicated. Cells were then stimulated with the TNFR2-specific TNF mutein sTNF143N/145R in the presence or absence of mAb 80M2 for 30 min (*arrow*). The change in the metabolic activity was followed over time and is expressed as percentage acidification.

data were obtained using mouse fibroblasts transfected with wild type TNFR2 and TRAF2-EGFP (data not shown).

To study TRAF2 recruitment to TNFR2 after memTNF-like stimulation also at physiological TRAF2 levels, we assessed coimmunoprecipitation studies with endogenously expressed TRAF2. TNFR2 was immunoprecipitated from HeLa cells, stably expressing TNFR2, and the precipitates were investigated for TRAF2 by Western blotting. 15 and 30 min after stimulation of the cells with sTNF, only slightly enhanced TRAF2 amounts were detectable in the immunoprecipitates as compared with unstimulated cells (Fig. 4*B*). TRAF2 coimmunoprecipitation could not be blocked with a TNFR1-specific antagonistic Fab fragment (αTNFR1-Fab), capable of inhibiting TNF binding to TNFR1 (Fig. 4*B*). In parallel experiments, we used TNFR2-specific Cys-TNF143N/145R for a memTNF-like stimulation of TNFR2. Immmunoprecipitates from these cells contained significantly larger amounts of TRAF2 as compared with that obtained from sTNF-treated cells (Fig. 4*B*), confirming the data obtained by confocal microscopy (Fig. 4*A*). Together, these results show that also in wild type TNFR2, the enhanced signaling capacity of memTNF-like stimuli, like Cys-TNF143N/145R, is linked to an enhanced recruitment of adaptor proteins and not dependent on overexpression of these intracellular signaling molecules.

We verified that enhanced adaptor recruitment to wild type TNFR2, as depicted in Fig. 4, *A* and *B*, is in fact paralleled by an enhanced cellular response. To this, HeLa80 cells and KYM-1 cells, known to activate NF-κB after appropriate stimulation via TNFR2 (16, 20), were examined for the activation of NF-κB by electrophoretic mobility shift assay. Nuclear translocation and DNA binding of NF-κB was determined after 30 min of receptor stimulation (*i.e.* within the same time range also investigated in the confocal microscopy experiments). These experiments revealed a similar stimulatory capacity of TNFR1 and TNFR2 for both cell lines (Fig. 5*A*). The TNFR2-selective sTNF143N/145R on its own showed only a marginal, if any, capacity to activate NF-κB, whereas the TNFR2-selective Cys-TNF143N/145R possessed an intermediate stimulatory capacity. This confirms that in HeLa and KYM-1 cells a memTNF-like activity is mandatory for full activation of TNFR2, whereas sTNF is sufficient to fully activate TNFR1. Finally, in TNFR2-expressing HeLa cells the overall metabolic response to a selective stimulation of TNFR2 by sTNF in the absence and presence of 80M2 was determined using a microphysiometer. The results confirm a strong cellular (*i.e.* metabolic) response initiated by TNFR2 only when stimulated with sTNF143N/145R in the presence of 80M2 (Fig. 5*B*).

Ligand Association and Dissociation Studies—In a previous

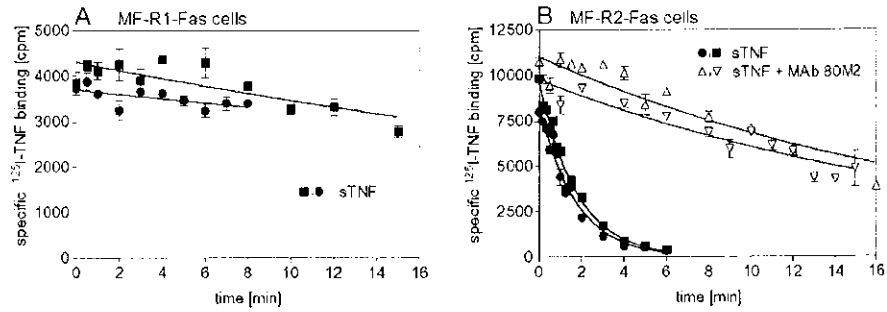

FIG. 6. **Time course of TNF dissociation at 37 °C.** MF-R1-Fas (*A*) and MF-R2-Fas (*B*) cells were incubated for 1 h with 10 ng/ml ^{125}I-TNF at 4 °C in the presence or absence of mAb 80M2 (2 μg/ml). Dissociation of the radiolabeled ligand was measured at 37 °C in the presence of 2 μg/ml unlabeled sTNF. Nonspecific binding determined in the presence of a 200-fold excess of unlabeled sTNF was less than 5% of total binding and has been subtracted. Half-lives of the TNF-receptor complexes were calculated from exponential decay curves.

publication, we showed that the enhanced signaling capacity of sTNF in the presence of 80M2, representing a memTNF-like stimulus, correlates with the stabilization of ligand-receptor complexes due to a strongly reduced dissociation rate (10). Using MF-R1-Fas and MF-R2-Fas cells, respectively, association and dissociation studies with iodinated sTNF at 37 °C were performed. The association kinetics of iodinated sTNF to both receptors were rapid and similar, with half-maximum binding after about 2 min for TNFR1-Fas and about 1 min for TNFR2-Fas at a ligand concentration of 0.3 nM (data not shown). The presence of the antibody 80M2 did not significantly change ligand association kinetics of TNFR2 (k_{on}(sTNF) = $3.0 \cdot 10^9$ M^{-1} min^{-1} and k_{on}(sTNF + 80M2) = $2.5 \cdot 10^9$ M^{-1} min^{-1}). To study ligand dissociation, untreated MF-R1-Fas and MF-R2-Fas cells, as well as 80M2-pretreated MF-R2-Fas cells, were incubated with 0.2 nM iodinated sTNF for 1 h on ice to allow receptor complex formation. The temperature was then shifted to 37 °C, and in the presence of a 200-fold excess of unlabeled sTNF, the release of the radiolabeled ligand was followed. Fig. 6*A* shows that sTNF dissociates only slowly from MF-R1-Fas cells (half-life = 32 min). In contrast, sTNF·TNFR2-Fas complexes have a low half-life of about 1.2 min, whereas 80M2 pretreatment stabilizes ligand·TNFR2-Fas complexes more than 10-fold (half-life = 14.5 min). These association and dissociation data are in good agreement with the results obtained with wild type TNF receptors (10). Accordingly, also the dissociation constants (K_d values) at 37 °C, as calculated for the chimeric receptors, are very similar to those values determined for the wild type TNF receptors (29 pM for TNFR1-Fas, 19 pM for TNFR1; 286 pM for TNFR2-Fas, 420 pM for TNFR2) (26). In summary, we show that the stability of ligand-receptor complexes correlates with the formation of the RISC and signal capacities of TNFR1-Fas and TNFR2-Fas chimeras.

DISCUSSION

In contrast to TNFR1, which is equally well activated by both sTNF and memTNF, TNFR2 can only be efficiently activated by memTNF. To address the molecular mechanisms underlying these different bioactivities, we have constructed receptor chimeras containing the extracellular domains of the TNF receptors fused to the intracellular part of Fas. In the present study, we demonstrate that these chimeric receptors exhibit identical activation requirements regarding the two TNF forms as the wild type TNF receptors. Moreover, our data suggest that the half-life of TNF·TNFR complexes becomes translated into the efficiency of intracellular adaptor recruitment, thus controlling the intensity of the transmitted signal.

Experimental systems quantitatively assessing the signal capacity of cell surface expressed memTNF are difficult to handle. We therefore used available tools that mimic memTNF signaling. We have recently described the TNFR2-specific monoclonal antibody 80M2 that, in combination with sTNF, induces intracellular signals comparable with the transmembrane form of TNF (10). Furthermore, we have utilized muteins of sTNF that form intermolecular disulfide bonds via an N-terminal cysteine residue (Cys-TNF and the receptor-specific derivatives Cys-TNF32W/86T and Cys-TNF143N/145R), resulting in the formation of secondary cross-linked TNF trimers. These TNF derivatives also show an enhanced signaling capacity via TNFR2 as compared with sTNF (Fig. 2*B*).[2]

To obtain a suitable molecular system for investigation of sTNF and memTNF action, we transferred the extracellular domains of the two TNF receptors to the cytoplasmic part of Fas, another TNF receptor family member. A simple exchange of the intracellular TNF receptor domains turned out to be inappropriate due to the constitutive cytotoxic activity of the intracellular region of TNFR1 (data not shown). To omit problems with endogenous TNF responsiveness, receptor chimeras were expressed in a fibroblast-derived cell line from TNFR1/TNFR2 double knockout mice. As expected, both chimeric receptors, TNFR1-Fas and TNFR2-Fas, could be expressed in quite high numbers in these cells, with about 15,000 and 45,000 TNF binding sites for MF-R1-Fas and -R2-Fas cells, respectively (data not shown). In the absence of any TNF receptor-specific stimulus, both cell lines proliferate well without indications of spontaneous apoptosis (data not shown).

In various studies on TNFR1- and Fas-expressing cells, conflicting results have been obtained regarding the involvement of membrane rafts or the requirement of internalization for signal initiation. Recently, the possible arrangement of Fas in lipid rafts was shown (31, 32), which may be true for type II but not for type I cells (9, 33). Furthermore, Fas signaling has been reported to be independent of receptor complex internalization (8, 9), whereas TNFR1 was not (8). In the present study, both TNFR-Fas chimeras have been expressed in reasonable receptor numbers in mouse fibroblasts and are therefore supposed to induce a very strong initial signal upon appropriate stimulation. This is in accordance with the rapid TNF-induced morphological changes, typical for apoptosis, within 1 (TNFR2-Fas) to 3 h (TNFR1-Fas). In addition, pretreatment of MF-R2-Fas cells with methyl-β-cyclodextrine, which disrupts lipid rafts by cholesterol depletion (34), or monodansylcadaverine, which blocks receptor internalization (8), did not affect cell death kinetics of MF-R2-Fas cells treated with sTNF plus 80M2 in a 3-h assay (data not shown). In agreement, using radioiodinated sTNF, we did not find significant internalization of ligand-receptor complexes within 30 min of incubation at 37 °C (<5% of TNFR1-Fas when stimulated with sTNF; <10% of TNFR2-Fas when stimulated with sTNF with or without 80M2; data not shown). Together, all of these data suggest that our cellular model displays a very rapid apoptotic response after appropriate TNF treatment that is largely independent of secondary processes following RISC formation and/or cofactors.

The Studies Performed with the TNFR-Fas Chimeras Allow Two Direct Conclusions—First, the differential response pattern of the two TNF receptors to sTNF and memTNF could be

fully transferred to the Fas signaling system (*i.e.* in the case of TNFR2 from a gene inductory pathway, acting via TRAF2 binding, to the apoptotic pathway of Fas, acting via FADD mediated caspase-8 activation). Identical patterns were found for three different cellular responses (*i.e.* the induction of apoptosis, activation of NF-κB, and activation of the mitogen-activated protein kinase JNK) (Fig. 2). These results clearly show that the responsiveness of the TNF receptors to the soluble *versus* the membrane bound form of TNF is independent of the particular intracellular signaling machinery. A direct consequence is that the decisive process, able to distinguish between sTNF and memTNF in the case of TNFR2 and TNFR2-Fas, is located upstream of the recruitment of cytoplasmic factors. This strongly argues for the existence of a general principle able to control the signaling strength of a given receptor within the TNF receptor family, which is not determined solely by the affinity of ligand/receptor interaction, since sTNF acting at saturating concentrations on TNFR2-Fas elicits only weak, if any, responses (Fig. 2, *B* and *E*).

Second, exogenously initiated cross-linking of ligand-receptor complexes is not necessary for induction of full signaling and receptor cluster formation. Cross-linking reagents like antibodies are commonly used for the efficient signal induction of Fas, TNFR2, or CD40 (11, 12, 16). Due to their multivalent nature, the treatment of cells with antibodies is paralleled by the formation of large receptor clusters (32, 35). However, the functional role of these clusters for signal initiation and strength has not been fully elucidated. In our studies, the efficient recruitment of FADD and TRAF2, respectively, is also paralleled by the formation of large receptor clusters, visible by colocalization of receptors with EGFP-tagged adaptor proteins (Figs. 3 (*B*, *F*, and *H*) and 4*A*) and in coimmunoprecipitation studies (Fig. 4*B*). This cluster formation always correlated with the particular signaling strength. In the case of TNFR2 and the TNFR2-derived Fas chimera, exogenously initiated cross-linking of ligand-receptor complexes was necessary for induction of full signaling and receptor cluster formation (Figs. 3 (*F* and *H*) and 4*A*). However, sTNF on its own is sufficient to initiate a strong intracellular signal via TNFR1-Fas, whereas in parallel triggering the formation of large receptor clusters (Figs. 2*A* and 3*B*). Since there are no observable differences in the efficiency of sTNF and memTNF upon TNFR1 stimulation (10),[3] the results argue against a mandatory role of an external, additional cross-linking agent for full activation of a given receptor. Recent observations by the group of Peter, demonstrating that formation of large Fas clusters is not directly dependent on the cross-linking properties of the stimulating agent (9), are consistent with these observations.

As discussed above, cluster formation occurs in parallel with enhanced signaling and is independent of the cytoplasmic part of the TNF receptors. It is therefore likely that the extracellular parts of ligand-bound receptors mediate cluster formation via additional interactions possibly involving supplementary molecules. Since the TNFR-Fas chimeras used in our studies contain the transmembrane parts of the respective TNF receptors, the possibility cannot be excluded that these domains are involved in receptor aggregate formation. When chimeric fusion proteins comprising the extracellular domain of the erythropoietin receptor and the intracellular part from TNFR2 were investigated, an exchange of the respective transmembrane domains had moderate effects on the signaling capacity (36). However, the present structural data of ligand trimerized receptors indicate that the transmembrane domains are unlikely to directly interact with each other between individual complexes (37, 38). More likely, a direct interaction of the ligated extracellular receptor domains might occur. Based on the dimeric crystal structure of the extracellular part of TNFR1, such a cluster formation has been already proposed by Naismith *et al.* (39). A possible candidate for receptor/receptor interaction is the recently defined preligand assembly domain, identified in both TNF receptors and in Fas, which is most likely present also in other members of the TNF receptor family (40).

What mechanisms beside additional cross-linking of receptor complexes could determine the enhanced signaling capability of memTNF? We propose that the stability of individual receptor-ligand complexes could be the important factor. Interactions of sTNF with TNFR1 and TNFR2 differ strongly in this regard. Whereas sTNF forms very stable complexes with TNFR1 (half-life = 33 min) (10) and TNFR1-Fas (half-life = 32 min) (Fig. 6*A*), ligation of TNFR2 (half-life = 1.1 min) (10) and TNFR2-Fas (half-life = 1.2 min) (Fig. 6*B*) occurs only very transiently. These differences are most likely inherent properties of the TNF/TNF receptor interactions rather than caused by different stoichiometries of complexes or subsequent steps like receptor cluster formation. This is evident from studies with TNF receptor-derived IgG fusion proteins that revealed very similar results (41). The exchange rates of radiolabeled sTNF complexed with TNFR-IgG fusion proteins showed a half-life of about 7 min for TNFR2-derived complexes, whereas TNFR1 complexes were extremely stable (half-life = 8 h) (41). Accordingly, the stability of individual ligand receptor complexes, most likely comprising three receptor molecules, would control subsequent steps leading to the formation of large, stable clusters, capable of effectively recruiting adaptors and initiating a strong intracellular signal. Certain conditions must be met, however. First, the initial sTNF binding must be rapid as compared with the subsequent steps. This holds true, since sTNF binding occurs very fast (26) and is mainly controlled by diffusion.[3] Second, receptor cluster formation should be dependent on preformed ligand-receptor complexes and should not occur spontaneously with unligated receptors. Most likely, this also holds true, since we observe no receptor patches directly after ligand binding on ice in our microscopy studies (Figs. 3 and 4). Third, formation of large receptor clusters must stabilize ligand binding in TNFR2 and TNFR2-Fas molecules. This also seems very reasonable, since integration of single receptor-ligand complexes into a lattice-like structure should inhibit dissociation of individual ligand-receptor complexes. We therefore suggest a causal relationship between the stability of individual receptor complexes and the efficiency of adaptor protein recruitment, the latter being directly translated into signaling strength. This principle might hold true for many, if not all, members of the TNF receptor family but is not necessarily restricted to these molecules.

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invitrogen

Material Safety Data Sheet

Revision Date: 20-Sep-2005

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18258
Product name ME DH5A COMPETENT CELLS

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	3-7

3. HAZARDS IDENTIFICATION

Emergency Overview

Irritating to eyes. Irritating to skin. Components of the product may be absorbed into the body through the skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects Eyes. Skin.

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C 189	°F No data available
Melting point/range	°C 18.4	°F No data available
Flash point	°C 94	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — Eyes. Skin.

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

Proper shipping name Not classified as dangerous within the meaning of transport regulations

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

Stratagene XL1-Blue Competent Cells, Catalog #200249

1. Product and company identification

Product name : **Stratagene XL1-Blue Competent Cells, Catalog #200249**

Part No. :

pUC18 Control Plasmid DNA	200231-42
1.42 M 2-Mercaptoethanol	210200-43
XL1-Blue Competent Cells	200236-41

Manufacturer / Supplier : Agilent Technologies, Inc.
1834 State Highway 71 West
Cedar Creek, TX 78612

Emergency telephone number : 1-800-894-1304

Use of the substance/preparation : Chemical Kit

Validation date : 01/09/2009

2. Hazards identification

Physical state :

pUC18 Control Plasmid DNA	Liquid.
1.42 M 2-Mercaptoethanol	Liquid.
XL1-Blue Competent Cells	Liquid.

Odor :

pUC18 Control Plasmid DNA	Not available.
1.42 M 2-Mercaptoethanol	Not available.
XL1-Blue Competent Cells	Not available.

OSHA/HCS status :

pUC18 Control Plasmid DNA	While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
1.42 M 2-Mercaptoethanol	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
XL1-Blue Competent Cells	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Emergency overview-Signal Word : WARNING !

Emergency overview-Label Statement :

pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

2 . Hazards identification

	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed. Irritating to eyes and skin. May cause sensitization by skin contact. Do not breathe vapor or mist. Do not ingest. Do not get on skin or clothing. Avoid contact with eyes. Wash thoroughly after handling.
	XL1-Blue Competent Cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling.
	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Routes of entry :	pUC18 Control Plasmid DNA	Eye contact. Ingestion.
	1.42 M 2-Mercaptoethanol	Dermal contact. Eye contact. Inhalation. Ingestion.
	XL1-Blue Competent Cells	Eye contact. Inhalation. Ingestion.
Potential acute health effects		
Eyes :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to eyes.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Skin :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed.
	XL1-Blue Competent Cells	Toxic if swallowed.
Medical conditions aggravated by over-exposure :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation.
	XL1-Blue Competent Cells	Repeated or prolonged exposure to the substance can produce target organs damage.
Over-exposure signs/symptoms :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
1.42 M 2-Mercaptoethanol		
2-Mercaptoethanol	60-24-2	10
XL1-Blue Competent Cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

Eye contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	:	pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.

4 . First aid measures

Ingestion	:	pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Notes to physician	:	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5 . Fire-fighting measures

Flammability of the product	:	pUC18 Control Plasmid DNA	Non-flammable.
		1.42 M 2-Mercaptoethanol	Non-flammable.
		XL1-Blue Competent Cells	Non-flammable.
Products of combustion	:	pUC18 Control Plasmid DNA	No specific data.
		1.42 M 2-Mercaptoethanol	Decomposition products may include the following materials: carbon oxides sulfur oxides
		XL1-Blue Competent Cells	Decomposition products may include the following materials: carbon oxides sulfur oxides halogenated compounds metal oxide/oxides

Extinguishing media

Suitable	:	pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
		1.42 M 2-Mercaptoethanol	Use an extinguishing agent suitable for the surrounding fire.
		XL1-Blue Competent Cells	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

Personal precautions	:	pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		1.42 M 2-Mercaptoethanol	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		XL1-Blue Competent Cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	:	pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		1.42 M 2-Mercaptoethanol	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		XL1-Blue Competent Cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up			
Small spill	:	pUC18 Control Plasmid DNA	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		1.42 M 2-Mercaptoethanol	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		XL1-Blue Competent Cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling	:	pUC18 Control Plasmid DNA	Wash thoroughly after handling.
		1.42 M 2-Mercaptoethanol	Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
		XL1-Blue Competent Cells	Do not ingest. Wash thoroughly after handling.

7 . Handling and storage

Storage : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
1.42 M 2-Mercaptoethanol	
2-Mercaptoethanol	**AIHA WEEL (United States, 1/2008).** TWA: 0.2 ppm 8 hour(s).
XL1-Blue Competent Cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust
Manganese dichloride	**ACGIH TLV (United States, 1/2008).** TWA: 0.2 mg/m³, (as Mn) 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** CEIL: 5 mg/m³, (as Mn) **NIOSH REL (United States, 12/2001).** TWA: 1 mg/m³, (as Mn) 10 hour(s). STEL: 3 mg/m³, (as Mn) 15 minute(s). **OSHA PEL (United States, 11/2006).** CEIL: 5 mg/m³, (as Mn)
Sucrose	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction **NIOSH REL (United States, 12/2001).** TWA: 10 mg/m³ 10 hour(s). Form: Total TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction **OSHA PEL (United States, 11/2006).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
Dimethyl sulfoxide	**AIHA WEEL (United States, 1/2008).** TWA: 250 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

8 . Exposure controls/personal protection

Skin : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Other protection : Not available.

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	: pUC18 Control Plasmid DNA	Liquid.
	1.42 M 2-Mercaptoethanol	Liquid.
	XL1-Blue Competent Cells	Liquid.
Flash point	: pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.
Color	: pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Not available.
Odor	: pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Not available.
pH	: pUC18 Control Plasmid DNA	Neutral.
	1.42 M 2-Mercaptoethanol	Neutral.
	XL1-Blue Competent Cells	Neutral.
Boiling/condensation point	: pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
	1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
	XL1-Blue Competent Cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F)
Melting/freezing point	: pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	1.42 M 2-Mercaptoethanol	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	XL1-Blue Competent Cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 3.02°C (37.4°F)

9 . Physical and chemical properties

Relative density	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Only known value: 1.1 (Water = 1) (2-Mercaptoethanol).
		XL1-Blue Competent Cells	Weighted average: 1.29 (Water = 1)
Vapor pressure	:	pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C)
		XL1-Blue Competent Cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.11 kPa (15.83 mm Hg) (at 20°C)
Vapor density	:	pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1)
		XL1-Blue Competent Cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.98 (Air = 1)
Evaporation rate	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	0.026 (Dimethyl sulfoxide) compared with Butyl acetate.

10 . Stability and reactivity

Stability and reactivity	:	The product is stable.
Incompatibility with various substances	:	Highly reactive or incompatible with the following materials: oxidizing materials and organic materials. Reactive or incompatible with the following materials: acids.
Hazardous decomposition products	:	pUC18 Control Plasmid DNA: Under normal conditions of storage and use, hazardous decomposition products should not be produced. 1.42 M 2-Mercaptoethanol: Under normal conditions of storage and use, hazardous decomposition products should not be produced. XL1-Blue Competent Cells: Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Conditions of reactivity - Flammability	:	Flammable in the presence of the following materials or conditions: open flames, sparks and static discharge.

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Dimethyl sulfoxide	LD50 Dermal	Rat	40 gm/kg	-
	LD50 Oral	Rat	14500 mg/kg	-
Sucrose	LD50 Oral	Rat	29700 mg/kg	-
Manganese dichloride	LD50 Oral	Rat	250 mg/kg	-
Glycerol	LD50 Dermal	Rabbit	>10 gm/kg	-
	LD50 Oral	Rat	12600 mg/kg	-
Potassium chloride	LD50 Oral	Rat	2600 mg/kg	-

Eyes	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to eyes.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.

11 . Toxicological information

Skin	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
		XL1-Blue Competent Cells	Toxic if swallowed.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
XL1-Blue Competent Cells						
Sucrose	A4	-	-	-	-	-

Potential chronic health effects

Chronic effects	:	Contains material that may cause target organ damage, based on animal data.
Carcinogenicity	:	No known significant effects or critical hazards.
Mutagenicity	:	No known significant effects or critical hazards.
Teratogenicity	:	No known significant effects or critical hazards.
Developmental effects	:	No known significant effects or critical hazards.
Fertility effects	:	No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation	:	No specific data.
Ingestion	:	No specific data.
Skin	:	No specific data.
Eyes	:	No specific data.

Target organs	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Other adverse effects	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

12 . Ecological information

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Dimethyl sulfoxide	-	Acute LC50 35 to 37 ml/L Fresh water	Fish	96 hours
	-	Acute LC50 34000000 ug/L Fresh water	Fish	96 hours
Manganese dichloride	-	Acute EC50 4700 ug/L Fresh water	Daphnia	48 hours
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Potassium chloride	-	Acute EC50 83000 ug/L Fresh water	Daphnia	48 hours
	-	Acute LC50 337 mg/L Fresh water	Daphnia	48 hours
	-	Acute LC50 435000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification :

pUC18 Control Plasmid DNA	Not regulated.
1.42 M 2-Mercaptoethanol	Toxic material Irritating material Sensitizing material
XL1-Blue Competent Cells	Toxic material Target organ effects

15 . Regulatory information

	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
U.S. Federal regulations :	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
	1.42 M 2-Mercaptoethanol	**United States inventory (TSCA 8b):** All components are listed or exempted.
	XL1-Blue Competent Cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
	pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
	1.42 M 2-Mercaptoethanol	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: 2-Mercaptoethanol **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard
	XL1-Blue Competent Cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose; Dimethyl sulfoxide **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard; Dimethyl sulfoxide: Immediate (acute) health hazard, Delayed (chronic) health hazard
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 307**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 307**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 311**: Edetic acid
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 311**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 311**: No products were found.

15 . Regulatory information

pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.

SARA 313

	Product name	CAS number	Concentration
Form R - Reporting requirements :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations : pUC18 Control Plasmid DNA

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the

15 . Regulatory information

components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

1.42 M 2-Mercaptoethanol	**Connecticut Carcinogen Reporting**: None of the components are listed. **Connecticut Hazardous Material Survey**: None of the components are listed. **Florida substances**: None of the components are listed. **Illinois Chemical Safety Act**: None of the components are listed. **Illinois Toxic Substances Disclosure to Employee Act**: None of the components are listed. **Louisiana Reporting**: None of the components are listed. **Louisiana Spill**: None of the components are listed. **Massachusetts Spill**: None of the components are listed. **Massachusetts Substances**: The following components are listed: 2-Mercaptoethanol **Michigan Critical Material**: None of the components are listed. **Minnesota Hazardous Substances**: None of the components are listed. **New Jersey Hazardous Substances**: None of the components are listed. **New Jersey Spill**: None of the components are listed. **New Jersey Toxic Catastrophe Prevention Act**: None of the components are listed. **New York Acutely Hazardous Substances**: None of the components are listed. **New York Toxic Chemical Release Reporting**: None of the components are listed. **Pennsylvania RTK Hazardous Substances**: The following components are listed: 2-Mercaptoethanol **Rhode Island Hazardous Substances**: None of the components are listed.
XL1-Blue Competent Cells	**Connecticut Carcinogen Reporting**: None of the components are listed. **Connecticut Hazardous Material Survey**: None of the components are listed. **Florida substances**: None of the components are listed. **Illinois Chemical Safety Act**: None of the components are listed. **Illinois Toxic Substances Disclosure to Employee Act**: None of the components are listed. **Louisiana Reporting**: None of the components are listed. **Louisiana Spill**: None of the components are listed. **Massachusetts Spill**: None of the components are listed. **Massachusetts Substances**: The following components are listed: Glycerol;Sucrose **Michigan Critical Material**: None of the components are listed. **Minnesota Hazardous Substances**: None of the components are listed. **New Jersey Hazardous Substances**: The following components are listed: Manganese dichloride **New Jersey Spill**: None of the components are listed. **New Jersey Toxic Catastrophe Prevention Act**: None of the components are listed. **New York Acutely Hazardous Substances**: None of the components are listed. **New York Toxic Chemical Release Reporting**: None of the

15 . Regulatory information

components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride;Sucrose
Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

Label requirements	: pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
	1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
	XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

Date of issue : 01/09/2009
Version : 1

Notice to reader

DISCLAIMER: This Material Safety Data Sheet is offered without charge to the clients of Agilent Technologies. Data is the most current available to Agilent Technologies at the time of preparation and is issued as a matter of information only, no warranty as to its accuracy or completeness is expressed or implied.

Indicates information that has changed from previously issued version.

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

Stratagene BL21 Competent Cells, Catalog # 200133

1. Product and company identification

Product name : **Stratagene BL21 Competent Cells, Catalog # 200133**

Part No. :

BL21 competent cells	200133-41
pUC18 Control Plasmid DNA	200231-42
1.42 M 2-Mercaptoethanol	210200-43

Manufacturer / Supplier : Agilent Technologies, Inc.
1834 State Highway 71 West
Cedar Creek, TX 78612

Emergency telephone number : 1-800-894-1304

Use of the substance/preparation : Chemical Kit

Validation date : 11/21/2008

MSDS'

2. Hazards identification

Physical state :

BL21 competent cells	Liquid.
pUC18 Control Plasmid DNA	Liquid.
1.42 M 2-Mercaptoethanol	Liquid.

Odor :

BL21 competent cells	Not available.
pUC18 Control Plasmid DNA	Not available.
1.42 M 2-Mercaptoethanol	Not available.

OSHA/HCS status :

BL21 competent cells	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
pUC18 Control Plasmid DNA	While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
1.42 M 2-Mercaptoethanol	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Emergency overview-Signal Word : WARNING !

Emergency overview-Label Statement :

BL21 competent cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION.
BL21 competent cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling.
pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
1.42 M 2-Mercaptoethanol	Toxic if swallowed. Irritating to eyes and skin. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.

2 . Hazards identification

Routes of entry	:	BL21 competent cells	Eye contact. Inhalation. Ingestion.
		pUC18 Control Plasmid DNA	Eye contact. Ingestion.
		1.42 M 2-Mercaptoethanol	Eye contact. Ingestion.
Potential acute health effects			
Eyes	:	BL21 competent cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to eyes.
Skin	:	BL21 competent cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin.
Inhalation	:	BL21 competent cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
Ingestion	:	BL21 competent cells	Toxic if swallowed.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
Medical conditions aggravated by over-exposure	:	BL21 competent cells	Repeated or prolonged exposure to the substance can produce target organs damage.
		pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation.
Over-exposure signs/symptoms	:	BL21 competent cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
BL21 competent cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5
1.42 M 2-Mercaptoethanol		
2-Mercaptoethanol	60-24-2	10

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

Eye contact	:	BL21 competent cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	BL21 competent cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	:	BL21 competent cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
Ingestion	:	BL21 competent cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	BL21 competent cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
Notes to physician	:	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5 . Fire-fighting measures

Flammability of the product	:	BL21 competent cells	Non-flammable.
		pUC18 Control Plasmid DNA	Non-flammable.
		1.42 M 2-Mercaptoethanol	Non-flammable.
Products of combustion	:	BL21 competent cells	Decomposition products may include the following materials: carbon oxides halogenated compounds metal oxide/oxides
		pUC18 Control Plasmid DNA	No specific data.
		1.42 M 2-Mercaptoethanol	Decomposition products may include the following materials: carbon oxides sulfur oxides

Extinguishing media

Suitable	:	BL21 competent cells	Use an extinguishing agent suitable for the surrounding fire.
		pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
		1.42 M 2-Mercaptoethanol	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	BL21 competent cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	BL21 competent cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

Personal precautions	:	BL21 competent cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		1.42 M 2-Mercaptoethanol	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).

6 . Accidental release measures

Environmental precautions	:	BL21 competent cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		1.42 M 2-Mercaptoethanol	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up			
Small spill	:	BL21 competent cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		pUC18 Control Plasmid DNA	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		1.42 M 2-Mercaptoethanol	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling	:	BL21 competent cells	Do not ingest. Wash thoroughly after handling.
		pUC18 Control Plasmid DNA	Wash thoroughly after handling.
		1.42 M 2-Mercaptoethanol	Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
Storage	:	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.	

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
BL21 competent cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust
Manganese dichloride	**ACGIH TLV (United States, 1/2008).** TWA: 0.2 mg/m³, (as Mn) 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** CEIL: 5 mg/m³, (as Mn) **NIOSH REL (United States, 12/2001).**

8 . Exposure controls/personal protection

	TWA: 1 mg/m³, (as Mn) 10 hour(s). STEL: 3 mg/m³, (as Mn) 15 minute(s). **OSHA PEL (United States, 11/2006).** CEIL: 5 mg/m³, (as Mn)
Sucrose	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction **NIOSH REL (United States, 12/2001).** TWA: 10 mg/m³ 10 hour(s). Form: Total TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction **OSHA PEL (United States, 11/2006).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
Dimethyl sulfoxide	**AIHA WEEL (United States, 1/2008).** TWA: 250 ppm 8 hour(s).

1.42 M 2-Mercaptoethanol

2-Mercaptoethanol	**AIHA WEEL (United States, 1/2008).** TWA: 0.2 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

Skin : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Other protection : Not available.

Hygiene measures : Handle as biohazard material (Biosafety level 1).
Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state : BL21 competent cells Liquid.
pUC18 Control Plasmid DNA Liquid.
1.42 M 2-Mercaptoethanol Liquid.

9 . Physical and chemical properties

Flash point	:	BL21 competent cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
Color	:	BL21 competent cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
Odor	:	BL21 competent cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
pH	:	BL21 competent cells	Neutral.
		pUC18 Control Plasmid DNA	Neutral.
		1.42 M 2-Mercaptoethanol	Neutral.
Boiling/condensation point	:	BL21 competent cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 116.29°C (241.3°F)
		pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
		1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
Melting/freezing point	:	BL21 competent cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 1.7°C (35.1°F)
		pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
		1.42 M 2-Mercaptoethanol	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
Relative density	:	BL21 competent cells	Weighted average: 1.41 (Water = 1)
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Only known value: 1.1 (Water = 1) (2-Mercaptoethanol).
Vapor pressure	:	BL21 competent cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C)
Vapor density	:	BL21 competent cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.83 (Air = 1)
		pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1)

10 . Stability and reactivity

Stability and reactivity	:	The product is stable.	
Incompatibility with various substances	:	Reactive or incompatible with the following materials: oxidizing materials and acids.	
Hazardous decomposition products	:	BL21 competent cells	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
		pUC18 Control Plasmid DNA	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
		1.42 M 2-Mercaptoethanol	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

10 . Stability and reactivity

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Dimethyl sulfoxide	LD50 Dermal	Rat	40 gm/kg	-
	LD50 Oral	Rat	14500 mg/kg	-
Sucrose	LD50 Oral	Rat	29700 mg/kg	-
Manganese dichloride	LD50 Oral	Rat	250 mg/kg	-
Glycerol	LD50 Dermal	Rabbit	>10 gm/kg	-
	LD50 Oral	Rat	12600 mg/kg	-
Potassium chloride	LD50 Oral	Rat	2600 mg/kg	-

Eyes :
- BL21 competent cells — No known significant effects or critical hazards.
- pUC18 Control Plasmid DNA — No known significant effects or critical hazards.
- 1.42 M 2-Mercaptoethanol — Irritating to eyes.

Skin :
- BL21 competent cells — No known significant effects or critical hazards.
- pUC18 Control Plasmid DNA — No known significant effects or critical hazards.
- 1.42 M 2-Mercaptoethanol — Irritating to skin.

Inhalation :
- BL21 competent cells — No known significant effects or critical hazards.
- pUC18 Control Plasmid DNA — No known significant effects or critical hazards.
- 1.42 M 2-Mercaptoethanol — No known significant effects or critical hazards.

Ingestion :
- BL21 competent cells — Toxic if swallowed.
- pUC18 Control Plasmid DNA — No known significant effects or critical hazards.
- 1.42 M 2-Mercaptoethanol — Toxic if swallowed.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
BL21 competent cells						
Sucrose	A4	-	-	-	-	-

Potential chronic health effects

Chronic effects : Contains material that may cause target organ damage, based on animal data.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation : No specific data.

Ingestion : No specific data.

Skin : No specific data.

Eyes : No specific data.

Other adverse effects :
- BL21 competent cells — Not available.
- pUC18 Control Plasmid DNA — Not available.
- 1.42 M 2-Mercaptoethanol — Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Dimethyl sulfoxide	-	Acute LC50 35 to 37 ml/L Fresh water	Fish	96 hours
	-	Acute LC50 34000000 ug/L Fresh water	Fish	96 hours
Manganese dichloride	-	Acute EC50 4700 ug/L Fresh water	Daphnia	48 hours
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Potassium chloride	-	Acute EC50 83000 ug/L Fresh water	Daphnia	48 hours
	-	Acute LC50 337 mg/L Fresh water	Daphnia	48 hours
	-	Acute LC50 435000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT /IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification :

BL21 competent cells	Toxic material Target organ effects
pUC18 Control Plasmid DNA	Not regulated.
1.42 M 2-Mercaptoethanol	Toxic material Irritating material

15 . Regulatory information

U.S. Federal regulations :	BL21 competent cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
	1.42 M 2-Mercaptoethanol	**United States inventory (TSCA 8b):** All components are listed or exempted.
	BL21 competent cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard
	pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
	1.42 M 2-Mercaptoethanol	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: 2-Mercaptoethanol **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard
	BL21 competent cells	**Clean Water Act (CWA) 307**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 307**: No products were found.
	BL21 competent cells	**Clean Water Act (CWA) 311**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 311**: Edetic acid
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 311**: No products were found.
	BL21 competent cells	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
	BL21 competent cells	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.

15 . Regulatory information

	BL21 competent cells	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.

SARA 313

		Product name	CAS number	Concentration
Form R - Reporting requirements	:	**BL21 competent cells**		
		Manganese dichloride	7773-01-5	5 - 10
		Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification	:	**BL21 competent cells**		
		Manganese dichloride	7773-01-5	5 - 10
		Hexaamminecobalt trichloride	10534-89-1	0.1 - 1

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations : BL21 competent cells

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol;Sucrose
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: The following components are listed: Manganese dichloride
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride;Sucrose
Rhode Island Hazardous Substances: None of the components are listed.

pUC18 Control Plasmid DNA

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.

15 . Regulatory information

Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

1.42 M 2-Mercaptoethanol

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: 2-Mercaptoethanol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: 2-Mercaptoethanol
Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

Label requirements	:	BL21 competent cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
		pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
		1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION.
Date of issue	:	11/21/2008	
Version	:	1	

Notice to reader

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Indicates information that has changed from previously issued version.

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Bacteria

ATCC® Number: **BAA-1025™** Order this Item Price: **$205.00**

Organism:	*Escherichia coli* (Migula) Castellani and Chalmers
Designations:	BL21
Depositor:	J Bull
History:	J Bull I J Molineux
Biosafety Level:	1
Shipped:	freeze-dried
Growth Conditions:	ATCC medium129: Nutrient agar with 0.5% NaCl **Temperature:** 37.0°C Duration: aerobic
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	bacteriophage host (host for bacteriophages T3 and T7)
Related Products:	bacteriophage:ATCC BAA-1025-B1 bacteriophage:ATCC BAA-1025-B2

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Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the requesting institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for ordering information and to place an order for ATCC cultures and products.

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Patent Depository

ATCC® Number:	PTA-1798™ Order this Item	Price:	$200.00
Designation / Description:	Escherichia coli DH5alpha		
U.S. Patent Number:	7,354,755		
Biosafety Level:	1		
Shipped:	frozen		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

This material is cited in a U.S. and/or other Patent or Patent Application, and may not be used to infringe on the patent claims. ATCC is required to inform the Patent Depositor of the party to which the material was furnished.

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Fungi ,Yeasts and Yeast Genetic Stock

ATCC® Number: **201389™** Order this Item Price: **$155.00**

Organism:	*Saccharomyces cerevisiae* Meyen ex E.C. Hansen deposited as *Saccharomyces cerevisiae* Hansen, teleomorph
Alternate State:	*Candida robusta* Diddens et Lodder
Designations:	BY4742 [ATCC 4040004, YVC1]
Depositors:	JD Boeke
Biosafety Level:	1
Shipped:	frozen
Genotype/ORF/ Gene Name:	MATalpha his3delta1 leu2delta0 lys2delta0 ura3delta0 [21662] [53392]
Growth Conditions:	ATCC medium 1245: YEPD **Temperature:** 25.0°C
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transformation host [53392]
Mating Type:	alpha
Karyotype:	Ploidy: haploid
Comments:	Derived from S288C, ATCC 204508. parental strain for the International Systematic Saccharomyces cerevisiae Gene Disruption Project [21662]
Related Products:	genomic DNA: ATCC 201389D-5
Subcollection:	Yeasts
References:	21662: Brachmann CB, et al. Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14: 115-132, 1998. PubMed: 9483801 53392: Palmer CP, et al. A TRP homolog in Saccharomyces cerevisiae forms an intracellular Ca2+ -permeable channel in the yeast vacuolar membrane. Proc. Natl. Acad. Sci. USA 98: 7801-7805, 2001. PubMed: 11427713

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Info for Cell Line(s)

Cell Biology

ATCC® Number:	**CRL-1555™** Order this Item	Price:	**$279.00**

Designations:	A-431
Depositors:	DJ Giard, SA Aaronson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** skin **Tissue:** epidermis **Disease:** epidermoid carcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 9,13 D16S539: 12,14 D5S818: 12,13 D7S820: 10 THO1: 9 TPOX: 11 vWA: 15,17
Cytogenetic Analysis:	This is a hypertriploid human cell line. The modal chromosome number was 74 occurring in 36% of cells. The rate of cells with higher ploidies was 1.0%.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 0 PGM1, 1 PGM3, 1
Age:	85 years

Related Links

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BioProducts

- Cell, microbial and molecular genomics products for the life sciences

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- Bio-materials management; basic repository to complex partnership-level services

Gender:	female
Comments:	The epidermoid carcinoma cell line A-431, derived from an 85-year-old female, is one of a series of cell lines established from solid tumors by D.J. Giard, et al. [23218]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 derivative:ATCC CRL-2592

References:	23093: Faust JB, Meeker TC. Amplification and expression of the bcl-1 gene in human solid tumor cell lines. Cancer Res. 52: 2460-2463, 1992. PubMed: 1568216 23218: Giard DJ, et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. 51: 1417-1423, 1973. PubMed: 4357758 32507: Kovelman R, et al. Enhanced transcriptional activation by E2 proteins from the oncogenic human papillomaviruses. J. Virol. 70: 7549-7560, 1996. PubMed: 8892874 32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812 32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996. PubMed: 8552591 32912: Wizemann TM, et al. Peptide methionine sulfoxide reductase contributes to the maintenance of adhesins in three major pathogens. Proc. Natl. Acad. Sci. USA 93: 7985-7990, 1996. PubMed: 8755589

Return to Top

Cell Biology

ATCC® Number:	**CCL-185™** Order this Item	Price:	**$279.00**

Designations:	A549
Depositors:	M Lieber
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** lung **Disease:** carcinoma
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9.3 TPOX: 8,11 vWA: 14
Cytogenetic Analysis:	This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

Related Links

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Cell Micrograph

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Product Information Sheet

Isoenzymes:	G6PD, B
Age:	58 years
Gender:	male
Ethnicity:	Caucasian
Comments:	This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218] Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030] The cells are positive for keratin by immunoperoxidase staining.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 2 X 10(3) and 1 X 10(4) viable cells/cm2. Do not exceed 7 X 10(4) cels/cm2. 6. Incubate cultures at 37°C. **Interval:** Maintain cultures at a cell concentration between 6 X 10(3) and 6 X 10(4) cell/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase

Doubling Time:	about 22 hours
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020

References:

23218: Giard DJ, et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. 51: 1417-1423, 1973. PubMed: 4357758
27669: Mayr GA, Freimuth P. A single locus on human chromosome 21 directs the expression of a receptor for adenovirus type 2 in mouse A9 cells. J. Virol. 71: 412-418, 1997. PubMed: 8985365
27819: Goodrum FD, Ornelles DA. The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle. J. Virol. 71: 548-561, 1997. PubMed: 8985383
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32347: Horikami SM, et al. The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication. Virology 222: 383-390, 1996. PubMed: 8806522
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32373: Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260
32394: Fang R, Aust AE. Induction of ferritin synthesis in human lung epithelial cells treated with crocidolite asbestos. Arch. Biochem. Biophys. 340: 369-375, 1997. PubMed: 9143343
32488: Geiger T, et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug Des. 13: 35-45, 1998. PubMed: 9474241
32496: Evdokiou A, Cowled PA. Tumor-suppressive activity of the growth arrest-specific gene GAS1 in human tumor cell lines. Int. J. Cancer 75: 568-577, 1998. PubMed: 9466658
32511: Giavedoni LD, Yilma T. Construction and characterization of replication-competent simian immunodeficiency virus vectors that express gamma interferon. J. Virol. 70: 2247-2251, 1996. PubMed: 8642649
32514: Bartz SR, et al. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. 70: 2324-2331, 1996. PubMed: 8642659
32722: Garofalo R, et al. Transcriptional activation of the interleukin-8 gene by respiratory syncytial virus infection in alveolar epithelial cells: nuclear translocation of the RelA transcription factor as a mechanism producing airway mucosal inflammation. J. Virol. 70: 8773-8781, 1996. PubMed: 8971006
32758: Jamaluddin M, et al. Inducible translational regulation of

Return to Top

Cell Biology

ATCC® Number:	**CRL-1573™** Order this Item	Price:	**$279.00**

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19

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Cell, microbial and molecular genomics products for the life sciences

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Bio-materials management; basic repository to complex partnership-level services

Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus
Comments:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406] The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended. 6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2. **Subcultivation Ratio:** 1:10 to 1:20 weekly. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003 derivative: ATCC CRL-10852 derivative: ATCC CRL-12006 derivative: ATCC CRL-12007 derivative: ATCC CRL-12013 derivative: ATCC CRL-12479 derivative: ATCC CRL-2029 derivative: ATCC CRL-2368 purified DNA:ATCC CRL-1573D

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22699: Harrison T, et al. Host-range mutants of adenovirus type 5 defective for growth in HeLa cells. Virology 77: 319-329, 1977. PubMed: 841862
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32829: Ansieau S, et al. Tumor necrosis factor receptor-associated factor (TRAF)-1, TRAF-2, and TRAF-3 interact in vivo with the CD30 cytoplasmic domain: TRAF-2 mediates

Return to Top

Cell Biology

ATCC® Number:	**CCL-2™** Order this Item	Price:	**$279.00**

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18

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Cytogenetic Analysis:	Modal number = 82; range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of Ml, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 also available as Certified Reference Material, ATCC CRM-CCL-2 derivative:ATCC CCL-2.1 derivative:ATCC CCL-2.2 derivative:ATCC CCL-2.3

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26623: Fang X, et al. Lysophosphatidylcholine stimulates activator protein 1 and the c-Jun N-terminal kinase activity. J.

Return to Top

PRODUCT: HeLa Tet-Off® Cell Line

CATALOG No.
630905

LOT NUMBER
Specified on product label.

STORAGE CONDITIONS
- Store cells in liquid nitrogen (–196°C) or in –150°C freezer.
- Store FBS in –20°C freezer.

STORAGE MEDIUM
70% Tet System Approved FBS, 20% DMEM & 10% DMSO

SHELF LIFE
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS
Dry ice (–70°C)

FOR RESEARCH USE ONLY

DESCRIPTION:
Human cervical epithelioid carcinoma-derived cell line that expresses the tetracycline-controlled transactivator (tTA). An inducible, high-level gene expression system can be generated by stably transfecting these cells with a plasmid or infecting these cells with a virus that expresses the gene of interest under the control of a suitable Tet Response Element (TRE).

CELL TYPE INFORMATION & RECOMMENDED CELL CULTURE MEDIUM
See back page.

PACKAGE CONTENTS:
- 1 ml HeLa Tet-Off Cell Line (2.0 x 10^6 cells/tube)
- 0.5 ml CHO-AA8-Luc Tet-Off Control Cell Line (1.0 x 10^6 cells/tube)
- 50 ml Tet System Approved FBS

OTHER
- Tet Systems User Manual (PT3001-1)
- Tet Systems Protocol-at-a-Glance (PT3001-2)

QUALITY CONTROL DATA:

Functional Tests

HeLa Tet-Off Cells were transiently transfected by electroporation with pTRE-Luc. Luciferase activity in the presence and absence of 2 µg/ml doxycycline (Cat. No. 631311) was measured 48 hr later as described in the Tet Systems User Manual. Induction was observed to be at least 400-fold when cells were grown in media containing Tet System Approved FBS (Cat. No. 631101 or 631106).

Luciferase activity in the presence and absence of 2 µg/ml doxycycline was measured after 48 hr for the CHO-AA8-Luc Tet-Off Control Cell Line. Induction was found to be at least 1,000-fold when cells were grown in media containing Tet System Approved FBS (Cat. No. 631101 or 631106).

***Mycoplasma* Contamination Test**

These lots of the two cell lines were tested and found to be free of *Mycoplasma* contamination.

REFERENCE:
1. Gossen, M. & Bujard, H. (1992) Tight control of gene expression in mammalian cells by tetracycline responsive promoters. *Proc. Natl. Acad. Sci. USA* **89**:5547-5551.

APPROVED BY: _____

(PA641627)

CELL TYPE INFORMATION

HeLa Tet-Off Cell Line: Human cervical epithelioid carcinoma cell line transformed with pUHD15-1 and pSV2neo. This cell line is neomycin resistant.

CHO-AA8-Luc Tet-Off Control Cell Line: Chinese hamster ovary stably transformed with pUHD15-1, pSV2neo, pTRE-Luc, and pTK-Hyg. This cell line is neomycin and hygromycin resistant.

RECOMMENDED CELL CULTURE MEDIUM

HeLa Tet-Off Cell Line: 90% Dulbecco's Modified Eagle's Medium (DMEM), 10% Tet System Approved Fetal Bovine Serum (FBS), 4 mM L-glutamine, 100 µg/ml G418, 100 units/ml penicillin G sodium & 100 µg/ml streptomycin sulfate.

CHO-AA8-Luc Tet-Off Control Cell Line: 90% Eagle Minimum Essential Medium (alpha modification), 10% Tet System Approved Fetal Bovine Serum (FBS), 4 mM L-glutamine, 100 µg/ml G418, 100 units/ml penicillin G sodium, 100 µg/ml streptomycin sulfate & 100 µg/ml hygromycin B.

ADDITIONAL NOTES

We recommend that you titrate each new lot of hygromycin to determine its optimal concentration due to the lot-to-lot variation in antibiotic activity.

The doubling time for HeLa Tet-Off cells is approximately 20 hours during log phase.

The morphology of HeLa Tet-Off cells is elongated and adherent with 2-3 filopodia.

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Cell Biology

ATCC® Number:	**CCL-2.2™**	Order this Item	Price:	**$279.00**

Designations:	HeLa S3
Depositors:	TT Puck
Biosafety Level:	2 [Cells contain human papilloma virus (HPV-18)]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** cervix **Disease:** adenocarcinoma
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1955
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18
Cytogenetic Analysis:	A medium-sized metacentric marker is present in 100% of the cells. HeLa Markers: One copy of M1, one copy of M2, two copies of M3, and one copy of M4.
Isoenzymes:	G6PD, A
Age:	31 years
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y

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Comments:	HeLa S3 is a clonal derivative of the parent HeLa line (see ATCC CCL-2). S3 was cloned in 1955 by T.T. Puck, P.I. Marcus, and S.J. Cieciura. [22814] The HeLa S3 clone has been very useful in the clonal analysis of mammalian cell populations relating to chromosomal variation, cell nutrition, and plaque-forming ability. This line can be adapted to grow in suspension. [25952] The cells are positive for keratin by immunoperoxidase staining. A culture at approximately passage 400 was submitted to the American Type Culture Collection in February, 1972. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. [23180]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:10 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020

References:	22263: Chen TR. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. Cytogenet. Cell Genet. 48: 19-24, 1988. PubMed: 3180844 22766: Boshart M, et al. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 3: 1151-1157, 1984. PubMed: 6329740 22814: Puck TT, et al. Clonal growth of mammalian cells in vitro; growth characteristics of colonies from single HeLa cells with and without a feeder layer. J. Exp. Med. 103: 273-283, 1956. PubMed: 13286432 23180: Yee C, et al. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am. J. Pathol. 119: 361-366, 1985. PubMed: 2990217 25929: Puck TT, Marcus PI. A rapid method for viable cell titration and clone production with HeLa cells in tissue culture: the use of x-irradiated cells to supply conditioning factors. Proc. Natl. Acad. Sci. USA 41: 432-437, 1955. 25932: . . J. Exp. Med. 104: 427-434, 1956. 25934: . . Methods Enzymol. 5: 90-119, 1962. 25952: Darnell JE Jr., et al. The effect of cell population density on the amino acid requirements for poliovirus synthesis in HeLa cells. J. Exp. Med. 110: 445-450, 1959. PubMed: 13814142 25953: Cohen EP, Eagle H. A simplified chemostat for the growth of mammalian cells: characteristics of cell growth in continuous culture. J. Exp. Med. 113: 467-474, 1961. 25957: Darnell JE Jr., Sawyer TK. Variation in plaque-forming ability among parental and clonal strains of HeLa cells. Virology 8: 223-229, 1959. PubMed: 13669339 25959: Sato G, et al. Molecular growth requirements of single mammalian cells. Science 126: 461-464, 1957. PubMed: 13486039 32358: Soares K, et al. cis-Acting elements involved in transcriptional regulation of the herpes simples virus type 1 latency-associated promoter 1 (LAP1) in vitro and in vivo. J. Virol. 70: 5384-5394, 1996. PubMed: 8764049 32524: Chang YE, et al. Properties of the protein encoded by the UL32 open reading frame of herpes simplex virus 1. J. Virol. 70: 3938-3946, 1996. PubMed: 8648731 32966: Jiang BH, et al. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. Am. J. Physiol. 271: C1172-C1180, 1996. PubMed: 8897823 33006: Genuario RR, Perry RP. The GA-binding protein can serve as both an activator and repressor of ribosomal protein gene transcription. J. Biol. Chem. 271: 4388-4395, 1996. PubMed: 8626789 33032: Ladner RD, et al. Identification of a consensus cyclin-dependent kinase phosphorylation site unique to the nuclear form of human deoxyuridine triphosphate nucleotidohydrolase. J. Biol. Chem. 271: 7752-7757, 1996. PubMed: 8631817 33033: Ladner RD, et al. Characterization of distinct nuclear

Return to Top

Cell Biology

ATCC® Number:	**TIB-152™** Order this Item	Price:	**$279.00**

Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human) lymphoblast
Morphology:	PHOTO
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.
Gender:	male

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Comments:	This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609] The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530] Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609] The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml. **Interval:** Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-1990 derivative:ATCC CRL-2063 derivative:ATCC TIB-153

References:	1609: Weiss A, et al. The role of T3 surface molecules in the activation of human T cells: a two-stimulus requirement for IL-2 production reflects events occurring at a pre-translational level. J. Immunol. 133: 123-128, 1984. PubMed: 6327821 23430: Gillis S, Watson J. Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2-producing human leukemia T cell line. J. Exp. Med. 152: 1709-1719, 1980. PubMed: 6778951 32253: Berninghausen O, Leippe M. Necrosis versus apoptosis as the mechanism of target cell death induced by Entamoeba histolytica. Infect. Immun. 65: 3615-3621, 1997. PubMed: 9284127 32368: Churchill MJ, et al. The rev-responsive element negatively regulates human immunodeficiency virus type 1 env mRNA expression in primate cells. J. Virol. 70: 5786-5790, 1996. PubMed: 8709194 32396: Kolanus W, et al. alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule. Cell 86: 233-242, 1996. PubMed: 8706128 32446: Gan W, Rhoads RE. Internal initiation of translation directed by the 5'-untranslated region of the mRNA for eIF4G, a factor involved in the picornavirus-induced switch from cap-dependent to internal initiation. J. Biol. Chem. 271: 623-626, 1996. PubMed: 8557663 32561: Tiffany HL, et al. Enhanced expression of the eosinophil-derived neurotoin ribonuclease (RNS2) gene requires interaction between the promoter and intron. J. Biol. Chem. 271: 12387-12393, 1996. PubMed: 8647842 32704: Chan YJ, et al. Synergistic interactions between overlapping binding sites for the serum response factor and ELK-1 proteins mediate both basal enhancement and phorbol ester responsiveness of primate cytomegalovirus. J. Virol. 70: 8590-8605, 1996. PubMed: 8970984 32755: Kung SH, Medveczky PG. Identification of a herpesvirus saimiri cis-acting DNA fragment that permits stable replication of episomes in transformed T cells. J. Virol. 70: 1738-1744, 1996. PubMed: 8627695 32796: Bloom TJ, Beavo JA. Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. Proc. Natl. Acad. Sci. USA 93: 14188-14192, 1996. PubMed: 8943082 32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: 8855306 32904: Linette GP, et al. Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation. Proc. Natl. Acad. Sci. USA 93: 9545-9552, 1996. PubMed: 8790367 32913: Miranda L, et al. Isolation of the human PC6 gene encoding the putative host protease for HIV-1 gp160 processing

Return to Top

Cell Biology

ATCC® Number:	**CRL-1740™**	Order this Item	Price:	**$279.00**

Designations:	LNCaP clone FGC
Depositors:	JS Horoszewicz
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent, single cells and loosely attached clusters
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** left supraclavicular lymph node
Cellular Products:	human prostatic acid phosphatase; prostate specific antigen [21889]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	Distribution of this material for commercial purposes will require execution of a Non-exclusive License Agreement. At the time of placing an order, customers must send a request to licensing@ATCC .org. Orders will be shipped when Customer Service receives confirmation from our Licensing officer.
Isolation:	**Isolation date:** 1977
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	androgen receptor, positive; estrogen receptor, positive [23045]
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 10,12 D16S539: 11 D5S818: 11,12 D7S820: 9.1,10.3 THO1: 9 TPOX: 8,9 vWA: 16,18

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Cytogenetic Analysis:	This is a hypotetraploid human cell line. The modal chromosome number was 84, occurring in 22% of cells. However, cells with chromosome counts of 86 (20%) and 87 (18%) also occurred at high frequencies. The rate of cells with higher ploidies was 6.0%.
Age:	50 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	LNCaP clone FGC was isolated in 1977 by J.S. Horoszewicz, et al., from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old Caucasian male (blood type B+) with confirmed diagnosis of metastatic prostate carcinoma. [21889] These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production). [23045] The cells do not produce a uniform monolayer, but grow in clusters which should be broken apart by repeated pipetting when subcultures are prepared. They attach only lightly to the substrate, do not become confluent and rapidly acidify the medium. Growth is very slow. The cells should be allowed to incubate undisturbed for the first 48 hours after subculture. When flask cultures are shipped, the majority of the cells become detached from the flask and float in the medium. Upon receipt, incubate the flask (in the usual position for monolayer cultures) for 24 to 48 hours to allow the cells to re-attach. The medium can then be removed and replaced with fresh medium. If desired, the contents of the flask can be collected, centrifuged at 300 X g for 15 minutes, resuspended in 10 ml of medium and dispensed into a single flask.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Maintain cultures at a cell concentration between 1 X 10(4) and 2 X 10(5) cells/cm2. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	about 34 hours
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-10995 purified DNA:ATCC CRL-1740D

References:	21889: . Models for prostate cancer. 37New York: Liss; 1980. 22410: Gibas Z, et al. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet. Cytogenet. 11: 399-404, 1984. PubMed: 6584201 23045: Horoszewicz JS, et al. LNCaP model of human prostatic carcinoma. Cancer Res. 43: 1809-1818, 1983. PubMed: 6831420 32283: Hu SX, et al. Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression. Cancer Res. 57: 3339-3343, 1997. PubMed: 9269991 33090: Boffa LC, et al. Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence. J. Biol. Chem. 271: 13228-13233, 1996. PubMed: 8662737

Return to Top

Cell Biology

ATCC® Number:	**HTB-22™**	Order this Item	Price:	**$279.00**

Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	estrogen receptor, expressed
Antigen Expression:	Blood Type O; Rh+
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15

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Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1
Age:	69 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% . **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Subculturing:	**Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. **Note:** if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures. 1. Remove culture medium to a centrifuge tube. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant. 6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. 7. Incubate cultures at 37C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	29 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 purified DNA:ATCC HTB-22D purified RNA:ATCC HTB-22R 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X

References:

21405: Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943-945, 1985. PubMed: 3933111
22871: Takahashi K, Suzuki K. Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7 cells. Int. J. Cancer 55: 453-458, 1993. PubMed: 8375929
23046: Brandes LJ, Hermonat MW. Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol. Cancer Res. 43: 2831-2835, 1983. PubMed: 6850594
23079: Lan MS, et al. Polypeptide core of a human pancreatic tumor mucin antigen. Cancer Res. 50: 2997-3001, 1990. PubMed: 2334903
23107: Pratt SE, Pollak MN. Estrogen and antiestrogen modulation of MCF7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media. Cancer Res. 53: 5193-5198, 1993. PubMed: 7693333
23113: Huguet EL, et al. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. Cancer Res. 54: 2615-2621, 1994. PubMed: 8168088
23217: Soule HD, et al. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 51: 1409-1416, 1973. PubMed: 4357757
25065: Bellet D, et al. Malignant transformation of nontrophoblastic cells is associated with the expression of chorionic gonadotropin beta genes normally transcribed in trophoblastic cells. Cancer Res. 57: 516-523, 1997. PubMed: 9012484
32275: Littlewood-Evans AJ, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. Cancer Res. 57: 5386-5390, 1997. PubMed: 9393764
32278: Komarova EA, et al. Intracellular localization of p53 tumor suppressor protein in gamma-irradiated cells is cell cycle regulated and determined by the nucleus. Cancer Res. 57: 5217-5220, 1997. PubMed: 9393737
32285: van Dijk MA, et al. A functional assay in yeas for the human estrogen receptor displays wild-type and variant estrogen receptor messenger RNAs present in breast carcinoma. Cancer Res. 57: 3478-3485, 1997. PubMed: 9270016
32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029
32344: Umekita Y, et al. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996. PubMed: 8876218
32467: Zamora-Leon SP, et al. Expression of the fructose transporter GLUT5 in human breast cancer. Proc. Natl. Acad.

Return to Top

Cell Biology

ATCC® Number:	CRL-1469™ Order this Item	Price:	$279.00

Designations:	PANC-1
Depositors:	M Lieber
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** pancreas **Tissue:** duct **Disease:** epithelioid carcinoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 11 D16S539: 11 D5S818: 11,13 D7S820: 8,10 THO1: 7,8 TPOX: 8,11 vWA: 15
Cytogenetic Analysis:	Chromosome studies indicate a modal number of 63 with 3 distinct marker chromosomes and a small ring chromosome. This is a hypertriploid human cell line. The modal chromosome number was 61, occurring in 32% of cells., However, cells with 63 chromosomes also occurred at a high frequency (22%). The rate of cells with higher ploidies was 8.5%.
Isoenzymes:	G6PD, B
Age:	56 years
Gender:	male
Ethnicity:	Caucasian
Comments:	Growth is inhibited by 1 unit/ml L-asparaginase. The cells will grow in soft agar.

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Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.53mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	culture medium 95%; DMSO, 5%
Doubling Time:	52 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	22850: Lieber M, et al. Establishment of a continuous tumor-cell line (panc-1) from a human carcinoma of the exocrine pancreas. Int. J. Cancer 15: 741-747, 1975. PubMed: 1140870 22859: Wu MC, et al. Mechanism of sensitivity of cultured pancreatic carcinoma to asparaginase. Int. J. Cancer 22: 728-733, 1978. PubMed: 363626 23079: Lan MS, et al. Polypeptide core of a human pancreatic tumor mucin antigen. Cancer Res. 50: 2997-3001, 1990. PubMed: 2334903

Return to Top

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PRODUCT: Saos-2 Tet-Off® Cell Line

CATALOG No. 630911
(Not sold separately)

LOT NUMBER
Specified on product label

STORAGE CONDITIONS
Liquid nitrogen vapor phase

STORAGE MEDIUM
70% Tet System Approved FBS, 20% DMEM, and 10% DMSO

SHELF LIFE
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS
Dry ice (–70°C)

DESCRIPTION
Saos-2 Tet-Off is a human osteosarcoma-derived cell line that expresses the tetracycline-regulated transactivator Tet-Off (1). Inducible expression of any gene can be achieved by transfecting or transducing this cell line with a vector containing your gene of interest under the control of a tetracycline-responsive promoter. Expression is induced by the withdrawal of doxycycline (Dox) from the culture medium.

CELL TYPE INFORMATION & RECOMMENDED CELL CULTURE MEDIUM
See back of page.

PACKAGE CONTENTS
- 1 ml Saos-2 Tet-Off Cell Line (2.0×10^6 cells/tube)

OTHER
- Tet-Off and Tet-On Gene Expression Systems User Manual (PT3001-1)
- Tet Cell Lines Protocol-at-a-Glance (PT3001-2)

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

Functional Test
Saos-2 Tet-Off Cells were transiently transfected by electroporation with pTRE2-Luc. Luciferase activity in the presence and absence of 1 µg/ml doxycycline (Cat No. 631311) was measured 48 hr later as described in the Tet Systems User Manual. Induction was observed to be at least 30-fold when cells were grown in medium containing Clontech's Tet System Approved FBS.

Mycoplasma Contamination Test
This lot of cells has been tested and found to be free of mycoplasma contamination.

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APPROVED BY: _____

(PA993392)

CELL TYPE INFORMATION

Saos-2 Tet-Off is a human osteosarcoma-derived cell line stably transfected with pTet-Off (pUHD15-1neo). This cell line is G418 resistant.

RECOMMENDED CELL CULTURE MEDIUM

Grow the cells in 90% Dulbecco's Modified Eagle's Medium (DMEM), 10% Tet System Approved Fetal Bovine Serum (FBS), 4 mM L-glutamine, 100 µg/ml G418, 100 units/ml penicillin G sodium, and 100 µg/ml streptomycin sulfate, in the presence of 5% CO_2.

ADDITIONAL NOTES

The Saos-2 Tet-Off Cell Line exhibits hygromycin resistance. We therefore recommend use of puromycin resistance vectors (e.g., pTRE2 with pPUR or pTRE2-pur) for secondary transfections in this cell line.

REFERENCE

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci.* USA **89**(12):5547–5551.

Cell Biology

ATCC® Number:	**HTB-96™**	Order this Item	Price:	**$279.00**

Designations:	U-2 OS
Depositors:	Hellstrom
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** bone **Disease:** osteosarcoma
Cellular Products:	osteosacoma derived growth factor (ODGF)
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin-like growth factor I (IGF-I); insulin-like growth factor II (IGF II)
Antigen Expression:	Blood Type A; Rh+; HLA A2, Aw30, B12, Bw35, B40(+/-)
DNA Profile (STR):	Amelogenin: X CSF1PO: 13 D13S317: 13 D16S539: 11,12 D5S818: 11 D7S820: 11,12 THO1: 6,9.3 TPOX: 11,12 vWA: 14,18
Cytogenetic Analysis:	Cell line U-2 OS is chromosomally highly altered, with chromosome counts in the hypertriploid range. We did not find the hypodiploid cell population described by J. Ponten, et al., Instead, most of the population has slightly higher counts than first described. Very few normal chromosomes are present, but a high number of stable marker chromosomes are identified., Different chromosomal rearrangements involving the same chromosomes (N1, N7, N9, and N11 particularly), are seen. Twenty-two markers are found including: t(9qter--->9q21::1p36--->1p::?), 7p+, iso(17q), t(15q;?), 4q+, del(3)(q21), 5q(aberrant) and others. [22509]

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Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 2 PGM3, 1
Age:	15 years
Gender:	female
Ethnicity:	Caucasian
Comments:	J. Ponten and E. Saksela derived this line (originally 2T) in 1964 from a moderately differentiated sarcoma of the tibia of a 15 year old girl. Viruses were not detected during co-cultivation with WI-38 cells or in CF tests against SV40, RSV or adenoviruses. Mycoplasma contamination was detected and eliminated in 1972.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	recommended serum:ATCC 30-2020

References:	22237: Heldin CH, et al. A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. Nature 319: 511-514, 1986. PubMed: 3456080 22509: Ponten J, Saksela E. Two established in vitro cell lines from human mesenchymal tumours. Int. J. Cancer 2: 434-447, 1967. PubMed: 6081590 23011: Raile K, et al. Human osteosarcoma (U-2 OS) cells express both insulin-like growth factor-I (IGF-I) receptors and insulin-like growth factor-II/mannose-6- phosphate (IGF-II/M6P) receptors and synthesize IGF-II: autocrine growth stimulation by IGF-II via the IGF-I receptor. J. Cell. Physiol. 159: 531-541, 1994. PubMed: 8188767 32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029 32308: Moradpour D, et al. Characterization of cell lines allowing titghtly regulated expression of heapatitis C virus core protein. Virology 222: 51-63, 1996. PubMed: 8806487

Return to Top

Cell Biology

ATCC® Number:	**CL-173™** Order this Item	Price:	**$279.00**

Designations:	3T3-L1
Depositors:	Massachusetts Institute of Technology
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Cell Type:** fibroblast
Cellular Products:	triglycerides [3491]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin, expressed
Age:	embryo
Comments:	L1 is a continuous substrain of 3T3 (Swiss albino) developed through clonal isolation. The cells undergo a pre-adipose to adipose like conversion as they progress from a rapidly dividing to a confluent and contact inhibited state. A high serum content in the medium enhances fat accumulation [PubMed ID: 4426090]. Tested and found negative for ectromelia virus (mousepox). This line is also designated as ATCC CCL-92.1.ATCC CL-173 was deposited in 1974 without passage number information from the depositor. At the time of submission, ATCC prepared approximately 30 vials of seed stock at about 4 passages beyond the original depositor material (passage number: unknown +4).

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Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum.
Subculturing:	**Protocol:** Never allow culture to become completely confluent. 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. The recommended inoculum is 2 to 3 X 10(3) cells/sq. cm. Subculture before cultures become 70 to 80% confluent or when cells reach 5 to 6 X10(4) viable cells/sq. cm. 6. Incubate cultures at 37C. **Interval:** Every three days **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	14 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 formerly distributed as:ATCC CCL-92.1 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X Recommended serum: ATCC 30-2030

References:

886: Green H, Meuth M. An established pre-adipose cell line and its differentiation in culture. Cell 3: 127-133, 1974. PubMed: 4426090
3491: Green H. Triglyceride-accumulating clonal cell line. US Patent 4,003,789 dated Jan 18 1977
32373: Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260
32455: Scherer PE, et al. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proc. Natl. Acad. Sci. USA 93: 131-135, 1996. PubMed: 8552590
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Return to Top

Cell Biology

ATCC® Number:	**CRL-1772™**	Order this Item	Price:	**$279.00**

Designations:	C2C12
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	myoblast 
Source:	**Tissue:** muscle **Strain:** C3H **Cell Type:** myoblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Comments:	This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903] The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953] Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427] Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

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Subculturing:	**Protocol:** IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT. Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture. Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum. 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2. 6. Incubate cultures at 37°C. **Medium Renewal:** Every two to three days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020

References:	22903: Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524 22953: Blau HM, et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846 23427: Katagiri T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [published erratum appears in J Cell Biol 1995 Feb;128(4):following 713]. J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324 28236: Chow YH, et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336 32828: Kessler PD, et al. Gene delivery to skeletal muscle results in sustanined expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064 33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

Return to Top

Cell Biology

ATCC® Number:	**CRL-1720™**	Order this Item	Price:	**$379.00**

Designations:	F9
Depositors:	S Strickland
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	epithelial
Source:	**Organ:** testis **Strain:** 129 **Disease:** embryonal carcinoma; testicular teratoma
Cellular Products:	plasminogen activator; laminin; type IV collagen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Age:	embryo
Comments:	F9 cells can be stimulated to differentiate into parietal endoderm in the presence of retinoic acid and dibutyryl cyclic AMP (cAMP). Differentiating cells synthesize plasminogen activator, laminin and type IV collagen. cAMP is active only on cells that have been treated with retinoic acid. The cells maintain three copies of the beta 1 integrin gene. Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

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Subculturing:	**Protocol:** NOTE: culture vessels must be coated with 0.1% gelatin prior to use.To do so, cover the surface of the vessel with 0.1% gelatin (Difco) in sterile distilled water for 2 hours at 4C, then wash three times with sterile distilled water. Treated flasks and dishes can be stored at room temperature. 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new coated culture vessels. 6. Incubate cultures at 37°C. ***Subcultivation Ratio:*** *A subcultivation ratio of 1:10 is recommended* ***Medium Renewal:*** *Every 2 to 3 days*
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	1160: Strickland S, et al. Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. Cell 21: 347-355, 1980. PubMed: 6250719 1161: Strickland S, Mahdavi V. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell 15: 393-403, 1978. PubMed: 214238 23426: Stephens LE, et al. Targeted deletion of beta 1 integrins in F9 embryonal carcinoma cells affects morphological differentiation but not tissue-specific gene expression. J. Cell Biol. 123: 1607-1620, 1993. PubMed: 7504677 26151: Berstine EG, et al. Alkaline phosphatase activity in mouse teratoma. Proc. Natl. Acad. Sci. USA 70: 3899-3903, 1973. PubMed: 4521215 32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. J. Biol. Chem. 271: 24105-24114, 1996. PubMed: 8798649

Return to Top

Cell Biology

ATCC® Number:	**CRL-1658™** Order this Item	Price:	**$279.00**

Designations:	NIH/3T3
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Strain:** NIH/Swiss **Cell Type:** fibroblast fibroblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Murine leukemia virus
Age:	embryo
Comments:	The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457]. Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37C. . DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less. **Subcultivation Ratio:** Inoculate 3 to 5 X 10(3) cells/cm2 **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:	22370: Jainchill JL, et al. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. 4: 549-553, 1969. PubMed: 4311790 26133: Andersson P, et al. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. Cell 16: 63-75, 1979. PubMed: 84715 26134: Copeland NG, Cooper GM. Transfection by exogenous and endogenous murine retrovirus DNAs. Cell 16: 347-356, 1979. PubMed: 222457 28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321 32372: Berson JF, et al. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus tyep 1 strains. J. Virol. 70: 6288-6295, 1996. PubMed: 8709256 32478: Jones PL, et al. Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. Mol. Cell. Biol. 17: 6970-6981, 1997. PubMed: 9372929 32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. J. Virol. 70: 7921-7928, 1996. PubMed: 8892915 32522: Siess DC, et al. Exceptional fusogenicity of chinese hamster ovary cells with murine retrovirus suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process. J. Virol. 70: 3432-439, 1996. PubMed: 8648675 32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. J. Biol. Chem. 271: 24105-24114, 1996. PubMed: 8798649 32557: Medin JA, et al. Correction in trans for Fabry disease: expression, secretion, and uptake of alpha-galactosidase A in patient-derived cells driven by a high-titer recombinant retroviral vector. Proc. Natl. Acad. Sci. USA 93: 7917-7922, 1996. PubMed: 8755577 32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812 32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996. PubMed: 8552591 32702: Cranmer LD, et al. Identification, analysis, and evolutionary relationships of the putative murine cytomegalovirus homologs of the human cytomegalovirus UL82 (pp71) and UL83 (pp65) matrix phosphoproteins. J. Virol. 70: 7929-7939, 1996. PubMed: 8892916 32724: Shisler J, et al. Induction of susceptibility to tumor necrosis factor by E1A is dependent on binding to either p300

Return to Top

Cell Biology

ATCC® Number:	**CCL-92™**	Order this Item	Price:	**$279.00**

Designations:	3T3-Swiss albino
Depositors:	H Green
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Cell Type:** fibroblast
Cellular Products:	Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1962
Cytogenetic Analysis:	This is a hypertriploid mouse cell line. The modal chromosome number was 68 occurring in 30% of cells. The rate of cells with higher ploidies was 2.4%.
Age:	embryo
Comments:	The 3T3 cell line was established by G. Todaro and H. Green in 1962 from disaggregated Swiss mouse embryos. [5732] The cells are contact inhibited. A confluent monolayer yields 40000 cells/sq cm. Tested and found negative for ectromelia virus (mousepox). The cells should be grown in plastic flasks, they do not grow well on some types of glass surfaces. A saturation density of approximately 50000 cells per sq cm can be reached.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. **Temperature:** 37.0°C

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Bio-materials management; basic repository to complex partnership-level services

Subculturing:	**Protocol:** Never allow culture to become completely confluent. Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. For plates (50mm) use an inoculum of 3 X 10 exp5 cells per plate and subculture every 3 days. For 75 sq cm flasks use 4 X 10 exp5 cells per flask and subculture every 3 days. **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Doubling Time:	18 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2030 irradiated to be used as feeder cells:ATCC 48-X

References:

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26263: Todaro GJ, et al. Transformation of properties of an established cell line by SV40 and polyoma virus. Proc. Natl. Acad. Sci. USA 51: 66-73, 1964. PubMed: 14104605

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32500: Campbell M, et al. The simian foamy virus type 1 transcriptional transactivator (Tas) binds and activates an enhancer element in the gag gene. J. Virol. 70: 6847-6855, 1996. PubMed: 8794326

33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

Return to Top

Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$279.00**

Designations:	COS-7
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast 
Source:	**Organ:** kidney **Cell Type:** SV40 transformed
Cellular Products:	T antigen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 parental cell line:ATCC CCL-70 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X

References:

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32447: Fernandez LM, Puett D. Lys583 in the third extracellular loop of the lutropin/choriogonadotropin receptor is critical for signaling. J. Biol. Chem. 271: 925-930, 1996. PubMed: 8557706
32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614
32500: Campbell M, et al. The simian foamy virus type 1 transcriptional transactivator (Tas) binds and activates an enhancer element in the gag gene. J. Virol. 70: 6847-6855, 1996. PubMed: 8794326
32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. J. Virol. 70: 7921-7928, 1996. PubMed: 8892915
32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. J. Biol. Chem. 271: 24105-24114, 1996. PubMed: 8798649
32566: Dittrich E, et al. A di-leucine motif and an upstream serine in the interleukin-6 (IL-6) signal transducer gp130 mediate ligand-induced endocytosis and down-regulation of the IL-6 receptor. J. Biol. Chem. 271: 5487-5494, 1996. PubMed: 8621406
32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812
32720: Chen Y, et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005
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32861: Wright DA, et al. Association of human fas (CD95) with a ubiquitin-conjugating enzyme (UBC-FAP). J. Biol. Chem. 49: 31037-31043, 1996. PubMed: 8940097
32893: Zhang J, et al. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J. Biol. Chem. 271: 18302-18305, 1996. PubMed: 8702465
33011: Ozcelebi F, et al. Phosphorylation of cholecystokinin receptors expressed on chinese hamster ovary cells. J. Biol. Chem. 271: 3750-3755, 1996. PubMed: 8631990
33013: Gibson S, et al. Functional LCK is required for optimal CD28-mediated activation of the TEC family tyrosine kinase EMT/ITK. J. Biol. Chem. 271: 7079-7083, 1996. PubMed: 8636141
33016: Shaul PW, et al. Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. J. Biol. Chem. 271: 6518-6522, 1996. PubMed: 8626455
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Return to Top

-------- Original Message --------
Subject:[Fwd: Fwd: updated Biological Agent Registry Form (Litchfield)]
Date: Mon, 21 Mar 2011 16:25:25 -0400
From: Laszlo Gyenis <lgyenis@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: David Litchfield <litchfi@uwo.ca>

Hi Jennifer,

I am sending you the plasmid maps of pBI, pRc/CMV, pTRE, pEGFP and pcDNA3.1 vectors.
We are not using oncogenes.

Thank you, Laszlo

Info on Plasmid(s)

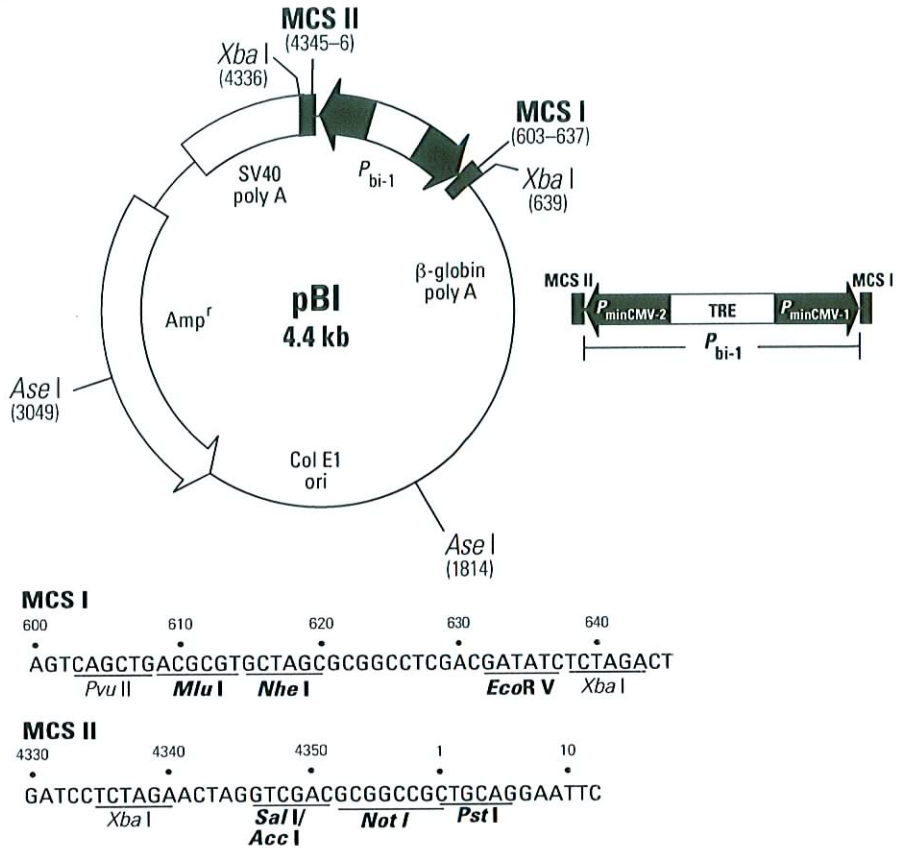
Vector Information

pBI Tet Vector Information

PT3070-5

GenBank Accession No.: U89932

Cat. No. 631006



Restriction Map and Multiple Cloning Site (MCS) of pBI Tet Vector. Unique restriction sites areshown in bold.

Description

The pBI Tet Vector is a response plasmid that can be used to express two genes of interest from one bidirectional tet-responsive promoter (P_{bi-1}; 1) in Clontech's Tet-On® and Tet-Off® Gene Expression Systems and Cell Lines (2). The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (3; Tet-Off) and Gossen *et al.* (4; Tet-On). The pBI Tet Vector contains the bidirectional promoter P_{bi-1} which is responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively. P_{bi-1} contains the Tet-responsive element (TRE), which consists of seven copies of the 42-bp tet operator sequence (*tetO*). The TRE element is between two minimal CMV promoters (P_{minCMV}), which lack the enhancer that is part of the complete CMV promoter. Consequently, P_{bi-1} is silent in the absence of binding of TetR or rTetR to the *tetO* sequences. $P_{minCMV-1}$ and $P_{minCMV-2}$ control the expression of two separate genes of interest. Note that the cloned inserts must have an initiation codon. In some cases, addition of a Kozak consensus ribosome binding site (5) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence.

Use

pBI allows the simultaneous regulation of two genes of interest by one central TRE. After a stable Tet-On or Tet-Off cell line has been established by transfecting with a tTA or rtTA regulator plasmid, pBI is cotransfected with pTK-Hyg (Cat No. 631750) to permit selection of a double-stable cell line which expresses both genes of interest. Alternatively, pPUR (Cat No. 631601) or another selection plasmid can be used. If this plasmid contains an enhancer element, as does pPUR, cointegration

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of pBI and the selection plasmid may lead to higher background expression. Double-stable, tet-responsive cell lines with pBI response constructs can be developed using the protocols described for pTRE response plasmids in the Tet Systems User Manual (PT3001-1).

Location of Features

- Multiple cloning site (MCS II): 4345–6
- P_{bi-1} Bidirectional Tet-responsive promoter: 12–568
  $P_{minCMV-2}$: 122–12
  Tet-responsive element (TRE): 128–439
  $P_{minCMV-1}$: 440–568
- Multiple cloning site (MCS I): 603–637
- Fragment containing the β-Globin poly A signal: 644–1811
- Col E1 origin of replication: 2012–2655
- Ampicillin resistance gene
  β-lactamase coding sequences: 3663–2803
- Fragment containing the SV40 poly A signal: 4328–3877

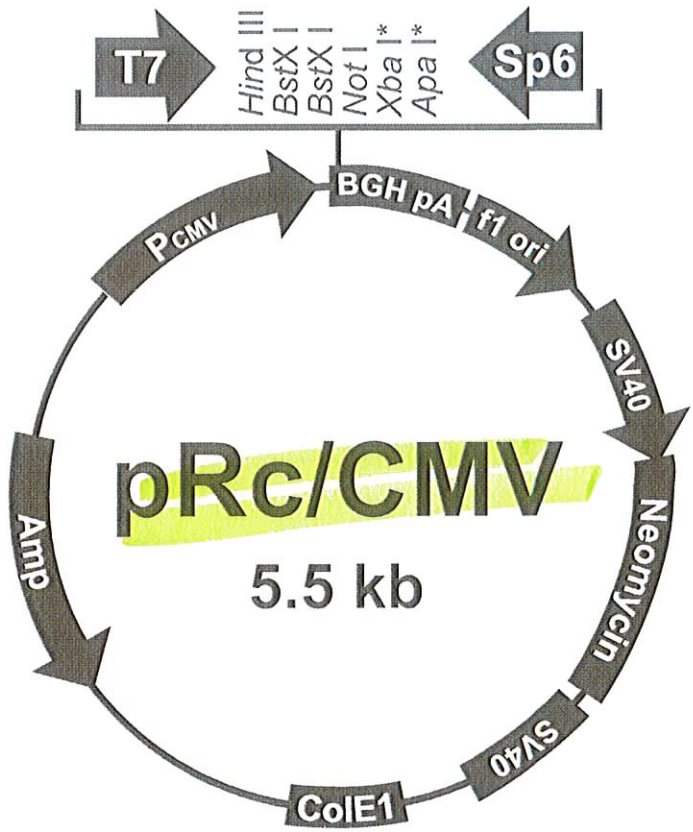
Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Baron, U., *et al.* (1995) *Nucleic Acids Res.* **17**:3605–3606.
2. Tet Expression Systems and Cell Lines (July 1996) *Clontechniques* **XI**(3):2–5.
3. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
4. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
5. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.



Comments for pRc/CMV:
5542 nucleotides

CMV promoter: bases 209-864
T7 promoter: bases 865-883
Polylinker: bases 890-995
Sp6 promoter: bases 1008-1017
BGH poly A signal: bases 1019-1250
f1 origin: bases 1306-1828
SV40 Promoter: bases 1890-2215
SV40 origin of replication: bases 2084-2169
Neo ORF: bases 2251-3045
SV40 poly A: bases 3219-3348
ColE1 origin: bases 3728-4400
Ampicillin resistance gene: bases 5406-4546
(complementary strand)

* There is an ATG upstream of the *Xba* I site.

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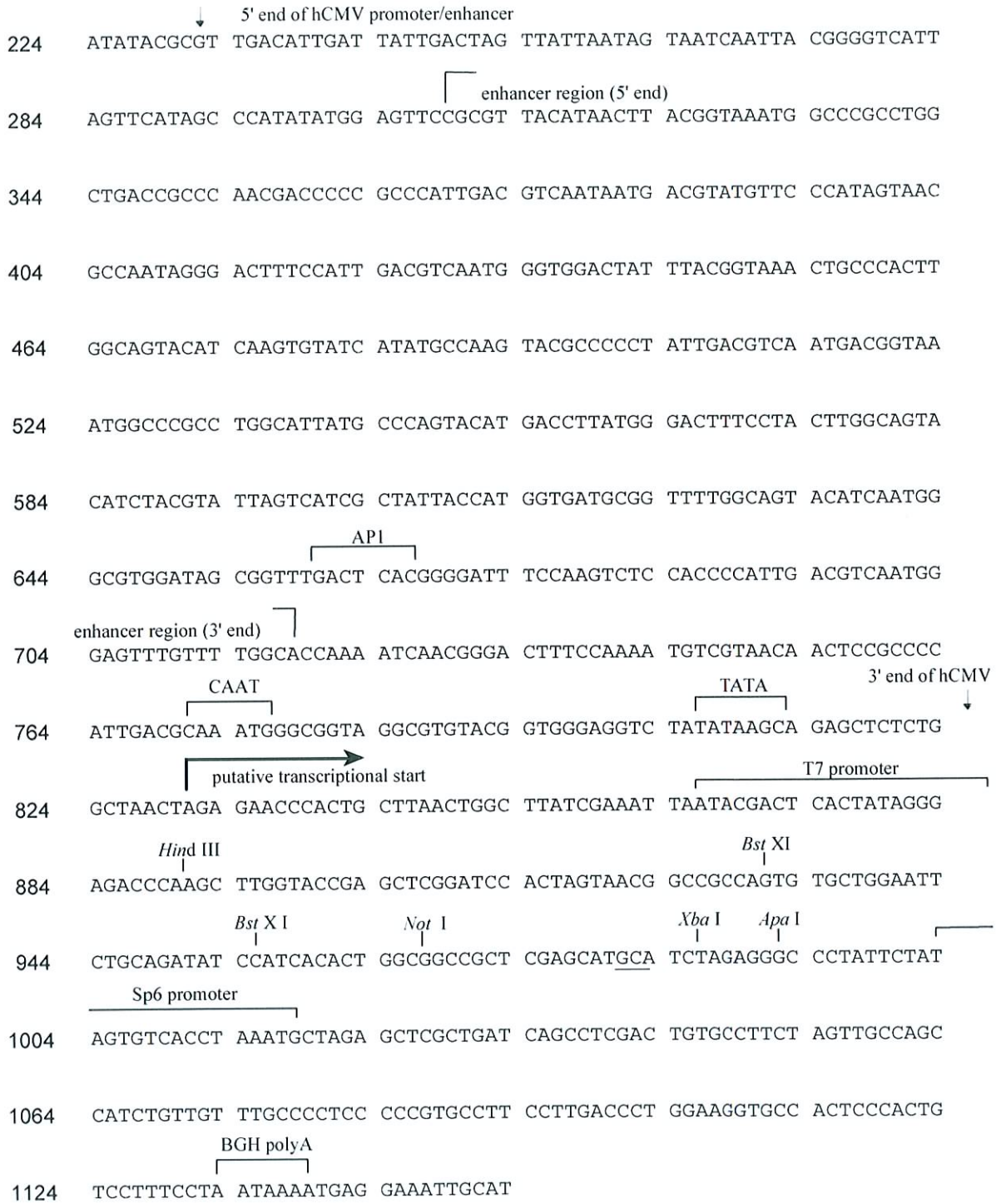
pRc/CMV

hCMV immediate early promoter
Polylinker
Primer binding sites
Bovine Growth Hormone polyA



NOTE: There is an ATG upstream of the *Xba* I and *Apa* I sites

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General Description

DNA pRc/CMV

Entire molecule length: 5542 bp

Restriction Map

Enzyme	# of cuts	Positions
AatI	1	2169
AatII	5	376 429 512 698 5542
Acc65I	2	897 1829
AccI	3	34 3358 5542
AciI	69	62 309 337 349 363 530 621(c) 654(c) 758 779(c) 926 966(c) 970 1253(c) 1277(c) 1326(c) 1340(c) 1343(c) 1371 1398 2018 2030 2039 2051 2061 2072 2118 2289 2352 2446(c) 2510(c) 2611(c) 2614(c) 2854 2894(c) 2899 2949(c) 2965 2991 3047(c) 3116 3119 3185(c) 3417 3520 3576(c) 3586(c) 3610 3653(c) 3660(c) 3681(c) 3772 3800 3927 3946 4067 4177(c) 4312 4321(c) 4683 4774 4965(c) 5011 5132(c) 5176 5253 5362 5461(c) 5508
AcsI	6	128 940 1727 1738 1841 3270
AcyI	9	373 426 509 695 2380 3082 3161 5157 5539
AflIII	2	229 3727
AluI	26	136 817 893 905 1025 1290 1457 1714 1837 2134 2188 2198 2486 2944 3235 3370 3392 3487 3551 3669 3895 4031 4288 4809 4909 4972
Alw44I	2	4041 5287
AlwI	23	13 16(c) 904(c) 917 1301(c) 1314 2224 2248 2559 2624(c)

		2805 3168(c) 3197 3345(c) 3358 4288(c) 4374(c) 4376 4472(c) 4473 4936(c) 5253 5257(c)
AlwNI	2	81 4143
AosI	2	2482 4842
ApaI	1	995
ApaLI	2	4041 5287
ApoI	6	128 940 1727 1738 1841 3270
AseI	5	258 864 3498 3557 4792
AsnI	5	258 864 3498 3557 4792
Asp700	1	5219
Asp718	2	897 1829
AspEI	1	4620
AspHI	9	819 907 1027 1839 2493 2683 4045 5206 5291
AspI	1	2498
AsuII	1	3062
AvaI	4	973 1297 2191 3363
AvaII	3	2896 4758 4980
AviII	2	2482 4842
AvrII	1	2170
BamHI	3	909 1306 3350
BanI	9	716 897 1109 1503 1829 2379 2414 3471 4568
BanII	8	819 907 995 1027 1262 1298 1839 2745
BbsI	1	1233
BbvI	18	188(c) 1374 2327(c) 2453 2495 2511(c) 2604(c) 3016 3244 3538(c) 3619(c) 3637(c) 4056(c) 4146 4149 4355 4658(c) 5049
BcgI	1	5159
BclI	2	1031 2221
BfaI	12	251 830 916 986 1020 1053 1391 2171 3305 4222 4475 4810
BglI	5	341 463 534 2123 4740
BglII	1	13
BlnI	1	2170
BmyI	15	819 907 995 1027 1262 1298 1839

		2326 2419 2493 2683 2745 4045 5206 5291
BpmI	3	3163(c) 3220 4690(c)
BpuAI	1	1233
BsaAI	3	591 1544 2684
BsaBI	2	954 2239
BsaHI	9	373 426 509 695 2380 3082 3161 5157 5539
BsaI	2	878(c) 4681(c)
BsaJI	15	611 1101 1821 1881 1890 1954 2077 2112 2121 2170 2191 2543 2812 3466 3887
BsaWI	4	2411 3933 4080 4911
BsiEI	7	926 970 2289 3643 4067 4990 5139
BsiHKAI	9	819 907 1027 1839 2493 2683 4045 5206 5291
BsiYI	15	300 894 1325 1651 1798 1895 2078 2361 2905 3186 3575 3749 3767 3933 4212
BsII	15	300 894 1325 1651 1798 1895 2078 2361 2905 3186 3575 3749 3767 3933 4212
BsmAI	5	685 878(c) 2222(c) 4681(c) 5457
BsmFI	9	426 577 745 1863(c) 1936(c) 2000(c) 2531 3063 3172
BsmI	1	3300(c)
Bsp120I	1	991
Bsp1286I	15	819 907 995 1027 1262 1298 1839 2326 2419 2493 2683 2745 4045 5206 5291
BspHI	2	4447 5455
BspMI	3	2267(c) 2648 3098
BspWI	38	198 341 463 495 534 627 651 850 975 1250 1274 1335 1337 1379 1406 1436 1892 1915 1987 2038 2117 2123 2371 2455

		2478 2617 2623 2740 2776 2823 3090 3471 3515 3599 3666 3780 4352 4740
BsrBI	7	972(c) 1400(c) 2993(c) 3047 3419(c) 3660 5461
BsrDI	3	2613 4681 4855(c)
BsrFI	5	1439 2699 2880 3163 4700
BsrI	18	75(c) 546(c) 854 929(c) 967 1633 2055(c) 2324 2525 3527(c) 4135 4148 4260(c) 4666(c) 4784(c) 4827(c) 5096 5266(c)
BssHII	1	2777
BstBI	1	3062
BstNI	12	341 534 1103 1883 1892 1939 1956 2767 3467 3755 3876 3889
BstUI	19	121 209 231 311 1316 1340 1360 1736 2446 2747 2779 3185 3574 3576 3774 4355 4685 5178 5510
BstXI	2	936 962
BstYI	14	13 909 1306 2216 2551 2797 3189 3350 4368 4379 4465 4477 5245 5262
CfoI	30	121 201 1318 1331 1340 1362 1388 1396 2374 2382 2446 2483 2749 2779 2781 3009 3185 3511 3576 3604 3637 3907 3974 4074 4248 4357 4750 4843 5180 5512
Cfr10I	5	1439 2699 2880 3163 4700
Csp45I	1	3062
Csp6I	12	44 214 469 494 549 582 633 790 898 1830 2685 5099
DdeI	11	40 110 181 1163 1272 2130 3043 4002 4411 4577

		5117
DpnI	30	7 15 23 911 1033 1308 2218 2223 2242 2553 2631 2712 2721 2799 3175 3191 3352 4295 4370 4381 4389 4467 4479 4584 4925 4943 4989 5247 5264 5300
DpnII	30	5 13 21 909 1031 1306 2216 2221 2240 2551 2629 2710 2719 2797 3173 3189 3350 4293 4368 4379 4387 4465 4477 4582 4923 4941 4987 5245 5262 5298
DraI	4	1769 4486 4505 5197
DraII	2	991 992
DraIII	1	1547
DrdI	3	1591 2407 3835
DsaI	3	611 2077 2812
DsaV	21	339 532 1101 1820 1881 1890 1937 1954 2190 2191 2382 2542 2765 3155 3465 3753 3874 3887 4105 4801 5152
EaeI	8	923 967 2286 2460 2851 2878 3566 5008
EagI	3	923 967 2286
Eam1105I	1	4620
EarI	4	2724(c) 2934(c) 3611 5415
Ecl136II	4	817 905 1025 1837
EclXI	3	923 967 2286
Eco57I	4	2526 2958 4275 5287(c)
EcoO109I	2	991 992
EcoRI	2	940 1841
EcoRII	12	339 532 1101 1881 1890 1937 1954 2765 3465 3753 3874 3887
EcoRV	2	952 2205
Fnu4HI	40	62 202 926 967 970 1327 1341 1363

		2118 2289 2341 2352 2442 2447 2484 2525 2612 2615 2618 2854 2950 2991 3005 3119 3233 3552 3633 3651 3654 3772 3927 4070 4135 4138 4344 4672 5011 5038 5133 5362
FnuDII	19	121 209 231 311 1316 1340 1360 1736 2446 2747 2779 3185 3574 3576 3774 4355 4685 5178 5510
FokI	10	1262 1457(c) 2021(c) 2247 2704 2729 3183 4586(c) 4767(c) 5054(c)
FspI	2	2482 4842
HaeII	5	1389 1397 2383 3605 3975
HaeIII	24	220 335 528 925 969 993 1552 1694 2111 2117 2126 2169 2288 2462 2853 2880 3568 3742 3753 3771 4205 4663 4743 5010
HgaI	6	777 3090 3169 3838 4416 5146(c)
HgiAI	9	819 907 1027 1839 2493 2683 4045 5206 5291
HhaI	30	121 201 1318 1331 1340 1362 1388 1396 2374 2382 2446 2483 2749 2779 2781 3009 3185 3511 3576 3604 3637 3907 3974 4074 4248 4357 4750 4843 5180 5512
HinP1I	30	119 199 1316 1329 1338 1360 1386 1394 2372 2380 2444 2481 2747 2777 2779 3007 3183 3509 3574 3602 3635 3905 3972 4072 4246 4355 4748 4841

		5178 5510
HincII	4	1 35 235 3359
HindII	4	1 35 235 3359
HindIII	1	891
HinfI	16	36 174 661 871 1592 1614 2865 2999 3051 3109 3146 3562 3627 3702 4098 4615
HpaII	23	1440 1821 2192 2285 2362 2384 2412 2543 2633 2700 2881 3156 3164 3445 3934 4081 4107 4297 4701 4735 4802 4912 5154
HphI	9	627 1001(c) 1544 2558(c) 4463(c) 4690(c) 5106 5312(c) 5347
ItaI	40	62 202 926 967 970 1327 1341 1363 2118 2289 2341 2352 2442 2447 2484 2525 2612 2615 2618 2854 2950 2991 3005 3119 3233 3552 3633 3651 3654 3772 3927 4070 4135 4138 4344 4672 5011 5038 5133 5362
KasI	1	2379
KpnI	2	901 1833
Ksp632I	4	2724(c) 2934(c) 3611 5415
MaeI	12	251 830 916 986 1020 1053 1391 2171 3305 4222 4475 4810
MaeII	17	373 385 426 509 590 695 1433 1543 1586 1598 1757 2496 2683 4430 4846 5219 5539
MaeIII	18	312 399 748 918 1007 1354 1366 2500 2806 3244 4083 4146 4262 4545 4876 4934 5087 5275
MamI	2	954 2239
MboI	30	5 13 21 909 1031

		1306 2216 2221 2240 2551 2629 2710 2719 2797 3173 3189 3350 4293 4368 4379 4387 4465 4477 4582 4923 4941 4987 5245 5262 5298
MboII	14	183 1238 1405(c) 1780(c) 2741 2951 3031(c) 3198(c) 3598(c) 4389 4460(c) 5215(c) 5293(c) 5402(c)
McrI	7	926 970 2289 3643 4067 4990 5139
MfeI	1	162
MluI	1	229
MluNI	1	2462
MnlI	33	96(c) 792(c) 982(c) 1048 1090 1135(c) 1210(c) 1267(c) 1293(c) 1517 2101(c) 2107(c) 2131 2137 2144(c) 2147(c) 2159(c) 2231(c) 2295(c) 2431(c) 2788(c) 2981 3187 3372 3576(c) 3626 3835(c) 3909 4159(c) 4559(c) 4640(c) 4787 4993
MscI	1	2462
MseI	23	70 132 258 846 864 1334 1605 1703 1720 1731 1743 1754 1768 3498 3557 4433 4485 4490 4504 4557 4792 4831 5196
MslI	6	616 1014 2817 4872 5031 5390
MspA1I	6	1290 2486 3551 4069 4314 5255
MspI	23	1440 1821 2192 2285 2362 2384 2412 2543 2633 2700 2881 3156 3164 3445 3934 4081 4107 4297 4701 4735 4802 4912 5154
MunI	1	162
MvaI	12	341 534 1103 1883

		1892 1939 1956 2767 3467 3755 3876 3889
MvnI	19	121 209 231 311 1316 1340 1360 1736 2446 2747 2779 3185 3574 3576 3774 4355 4685 5178 5510
MwoI	38	198 341 463 495 534 627 651 850 975 1250 1274 1335 1337 1379 1406 1436 1892 1915 1987 2038 2117 2123 2371 2455 2478 2617 2623 2740 2776 2823 3090 3471 3515 3599 3666 3780 4352 4740
NaeI	3	1441 2882 3165
NarI	1	2380
NciI	9	1822 2192 2193 2384 2544 3157 4107 4803 5154
NcoI	3	611 2077 2812
NdeI	1	485
NdeII	30	5 13 21 909 1031 1306 2216 2221 2240 2551 2629 2710 2719 2797 3173 3189 3350 4293 4368 4379 4387 4465 4477 4582 4923 4941 4987 5245 5262 5298
NgoMI	3	1439 2880 3163
NlaIII	23	171 555 615 982 1244 1918 1990 2081 2254 2599 2785 2816 2842 3198 3346 3385 3731 4451 4942 4952 5030 5066 5459
NlaIV	22	718 899 911 993 1111 1308 1484 1505 1831 1887 1960 2381 2416 3352 3473 3759 3798 4570 4664 4705 4916 5506
NotI	1	967

NruI	1	209
NsiI	3	984 1920 1992
NspI	6	982 1244 1918 1990 2785 3731
NspV	1	3062
PaeR7I	3	973 1297 3363
PleI	9	30(c) 655(c) 865(c) 1600 1608(c) 3045(c) 3621(c) 4106 4609(c)
Ppu10I	3	980 1916 1988
Psp1406I	3	1757 4846 5219
PstI	2	949 2433
PvuI	1	4990
PvuII	3	1290 2486 3551
RcaI	2	4447 5455
RsaI	12	45 215 470 495 550 583 634 791 899 1831 2686 5100
RsrII	1	2896
SacI	4	819 907 1027 1839
SalI	3	33 3357 5541
SapI	3	2724(c) 2934(c) 3611
Sau3AI	30	5 13 21 909 1031 1306 2216 2221 2240 2551 2629 2710 2719 2797 3173 3189 3350 4293 4368 4379 4387 4465 4477 4582 4923 4941 4987 5245 5262 5298
Sau96I	11	218 334 527 991 992 1550 2896 4662 4741 4758 4980
ScaI	1	5100
ScrFI	21	341 534 1103 1822 1883 1892 1939 1956 2192 2193 2384 2544 2767 3157 3467 3755 3876 3889 4107 4803 5154
SexAI	1	1937
SfaNI	19	48(c) 608(c) 991 1160 1240(c) 1479 1927 1999 2338(c) 2593(c) 2679 2743 2809(c) 3018 3272 3824 4876 5067(c) 5316
SfcI	8	877 945 1001 1321 2429 3992 4183 4861

SfiI	1	2123
SfuI	1	3062
SmaI	1	2193
SnaBI	1	591
SnoI	2	4041 5287
SpeI	2	250 915
SphI	5	982 1244 1918 1990 2785
SspI	3	1752 1773 5424
StuI	1	2169
StyI	4	611 2077 2170 2812
SwaI	1	1769
TaqI	19	34 858 974 1040 1298 1509 1839 2493 2649 2673 2709 2871 3062 3107 3358 3364 3827 5271 5542
TfiI	7	174 2865 2999 3109 3146 3562 3702
ThaI	19	121 209 231 311 1316 1340 1360 1736 2446 2747 2779 3185 3574 3576 3774 4355 4685 5178 5510
Tru9I	23	70 132 258 846 864 1334 1605 1703 1720 1731 1743 1754 1768 3498 3557 4433 4485 4490 4504 4557 4792 4831 5196
Tsp509I	20	128 162 269 861 940 1146 1727 1738 1764 1841 1924 1996 2088 3270 3407 3424 3499 4487 4793 5048
Tth111I	1	2498
XbaI	1	985
XcmI	1	846
XhoI	3	973 1297 3363
XhoII	14	13 909 1306 2216 2551 2797 3189 3350 4368 4379 4465 4477 5245 5262
XmaI	1	2191
XmaIII	3	923 967 2286
XmnI	1	5219

No cuts: AccIII, AflII, AgeI, AscI, BbrPI, BfrI, Bpu1102I, BseAI, BsgI, BsiWI, BspDI, BspEI, BsrGI, Bst1107I, BstEII, Bsu36I, CelII, ClaI, Eco47III, EcoNI, Esp3I, EspI, HpaI, KspI, MroI, NheI, PacI, PflMI, PinAI, PmaCI, PmeI, PmlI, PpuMI, SacII, SgrAI, SspBI, Van91I

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        10          20          30          40          50          60
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG

        70          80          90         100         110         120
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG

       130         140         150         160         170         180
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC

       190         200         210         220         230         240
TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT

       250         260         270         280         290         300
GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA

       310         320         330         340         350         360
TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC

       370         380         390         400         410         420
CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC

       430         440         450         460         470         480
ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT

       490         500         510         520         530         540
ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT

       550         560         570         580         590         600
ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA

       610         620         630         640         650         660
TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG

       670         680         690         700         710         720
ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC

       730         740         750         760         770         780
AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG

       790         800         810         820         830         840
GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
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       850         860         870         880         890         900
CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCCA AGCTTGGTAC

       910         920         930         940         950         960
CGAGCTCGGA TCCACTAGTA ACGGCCGCCA GTGTGCTGGA ATTCTGCAGA TATCCATCAC

       970         980         990        1000        1010        1020
ACTGGCGGCC GCTCGAGCAT GCATCTAGAG GGCCCTATTC TATAGTGTCA CCTAAATGCT

      1030        1040        1050        1060        1070        1080
AGAGCTCGCT GATCAGCCTC GACTGTGCCT TCTAGTTGCC AGCCATCTGT TGTTTGCCCC

      1090        1100        1110        1120        1130        1140
TCCCCCGTGC CTTCCTTGAC CCTGGAAGGT GCCACTCCCA CTGTCCTTTC CTAATAAAAT

      1150        1160        1170        1180        1190        1200
GAGGAAATTG CATCGCATTG TCTGAGTAGG TGTCATTCTA TTCTGGGGGG TGGGGTGGGG

      1210        1220        1230        1240        1250        1260
CAGGACAGCA AGGGGGAGGA TTGGGAAGAC AATAGCAGGC ATGCTGGGGA TGCGGTGGGC

      1270        1280        1290        1300        1310        1320
TCTATGGCTT CTGAGGCGGA AAGAACCAGC TGGGGCTCGA GGGGGGATCC CCACGCGCCC

      1330        1340        1350        1360        1370        1380
TGTAGCGGCG CATTAAGCGC GGCGGGTGTG GTGGTTACGC GCAGCGTGAC CGCTACACTT

      1390        1400        1410        1420        1430        1440
GCCAGCGCCC TAGCGCCCGC TCCTTTCGCT TTCTTCCCTT CCTTTCTCGC CACGTTCGCC

      1450        1460        1470        1480        1490        1500
GGCTTTCCCC GTCAAGCTCT AAATCGGGGC ATCCCTTTAG GGTTCCGATT TAGTGCTTTA

      1510        1520        1530        1540        1550        1560
CGGCACCTCG ACCCCAAAAA ACTTGATTAG GGTGATGGTT CACGTAGTGG GCCATCGCCC

      1570        1580        1590        1600        1610        1620
TGATAGACGG TTTTTCGCCC TTTGACGTTG GAGTCCACGT TCTTTAATAG TGGACTCTTG

      1630        1640        1650        1660        1670        1680
TTCCAAACTG GAACAACACT CAACCCTATC TCGGTCTATT CTTTTGATTT ATAAGGGATT

      1690        1700        1710        1720        1730        1740
```

```
TTGGGGATTT CGGCCTATTG GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT

      1750        1760        1770        1780        1790        1800
TTTAACAAAA TATTAACGTT TACAATTTAA ATATTTGCTT ATACAATCTT CCTGTTTTTG

      1810        1820        1830        1840        1850        1860
GGGCTTTTCT GATTATCAAC CGGGGTGGGT ACCGAGCTCG AATTCTGTGG AATGTGTGTC

      1870        1880        1890        1900        1910        1920
AGTTAGGGTG TGGAAAGTCC CCAGGCTCCC CAGGCAGGCA GAAGTATGCA AAGCATGCAT

      1930        1940        1950        1960        1970        1980
CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG

      1990        2000        2010        2020        2030        2040
CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG

      2050        2060        2070        2080        2090        2100
CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT

      2110        2120        2130        2140        2150        2160
TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT

      2170        2180        2190        2200        2210        2220
TTTGGAGGCC TAGGCTTTTG CAAAAAGCTC CCGGGAGCTT GGATATCCAT TTTCGGATCT

      2230        2240        2250        2260        2270        2280
GATCAAGAGA CAGGATGAGG ATCGTTTCGC ATGATTGAAC AAGATGGATT GCACGCAGGT

      2290        2300        2310        2320        2330        2340
TCTCCGGCCG CTTGGGTGGA GAGGCTATTC GGCTATGACT GGGCACAACA GACAATCGGC

      2350        2360        2370        2380        2390        2400
TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC GCCCGGTTCT TTTTGTCAAG

      2410        2420        2430        2440        2450        2460
ACCGACCTGT CCGGTGCCCT GAATGAACTG CAGGACGAGG CAGCGCGGCT ATCGTGGCTG

      2470        2480        2490        2500        2510        2520
GCCACGACGG GCGTTCCTTG CGCAGCTGTG CTCGACGTTG TCACTGAAGC GGGAAGGGAC

      2530        2540        2550        2560        2570        2580
TGGCTGCTAT TGGGCGAAGT GCCGGGGCAG GATCTCCTGT CATCTCACCT TGCTCCTGCC
```

```
      2590        2600        2610        2620        2630        2640
GAGAAAGTAT CCATCATGGC TGATGCAATG CGGCGGCTGC ATACGCTTGA TCCGGCTACC

      2650        2660        2670        2680        2690        2700
TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG CACGTACTCG GATGGAAGCC

      2710        2720        2730        2740        2750        2760
GGTCTTGTCG ATCAGGATGA TCTGGACGAA GAGCATCAGG GGCTCGCGCC AGCCGAACTG

      2770        2780        2790        2800        2810        2820
TTCGCCAGGC TCAAGGCGCG CATGCCCGAC GGCGAGGATC TCGTCGTGAC CCATGGCGAT

      2830        2840        2850        2860        2870        2880
GCCTGCTTGC CGAATATCAT GGTGGAAAAT GGCCGCTTTT CTGGATTCAT CGACTGTGGC

      2890        2900        2910        2920        2930        2940
CGGCTGGGTG TGGCGGACCG CTATCAGGAC ATAGCGTTGG CTACCCGTGA TATTGCTGAA

      2950        2960        2970        2980        2990        3000
GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTCGTGCTTT ACGGTATCGC CGCTCCCGAT

      3010        3020        3030        3040        3050        3060
TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT TCTGAGCGGG ACTCTGGGGT

      3070        3080        3090        3100        3110        3120
TCGAAATGAC CGACCAAGCG ACGCCCAACC TGCCATCACG AGATTTCGAT TCCACCGCCG

      3130        3140        3150        3160        3170        3180
CCTTCTATGA AAGGTTGGGC TTCGGAATCG TTTTCCGGGA CGCCGGCTGG ATGATCCTCC

      3190        3200        3210        3220        3230        3240
AGCGCGGGGA TCTCATGCTG GAGTTCTTCG CCCACCCCAA CTTGTTTATT GCAGCTTATA

      3250        3260        3270        3280        3290        3300
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      3310        3320        3330        3340        3350        3360
ATTCTAGTTG TGGTTTGTCC AAACTCATCA ATGTATCTTA TCATGTCTGG ATCCCGTCGA

      3370        3380        3390        3400        3410        3420
CCTCGAGAGC TTGGCGTAAT CATGGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT
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      3430        3440        3450        3460        3470        3480
CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT AAAGCCTGGG GTGCCTAATG

      3490        3500        3510        3520        3530        3540
AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC GCTTTCCAGT CGGGAAACCT

      3550        3560        3570        3580        3590        3600
GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCGTATTGG

      3610        3620        3630        3640        3650        3660
GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC

      3670        3680        3690        3700        3710        3720
GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG

      3730        3740        3750        3760        3770        3780
AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT

      3790        3800        3810        3820        3830        3840
GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA

      3850        3860        3870        3880        3890        3900
GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT

      3910        3920        3930        3940        3950        3960
CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC

      3970        3980        3990        4000        4010        4020
GGGAAGCGTG GCGCTTTCTC AATGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT

      4030        4040        4050        4060        4070        4080
TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC

      4090        4100        4110        4120        4130        4140
CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC

      4150        4160        4170        4180        4190        4200
CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG

      4210        4220        4230        4240        4250        4260
GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC

      4270        4280        4290        4300        4310        4320
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AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG

      4330        4340        4350        4360        4370        4380
CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA

      4390        4400        4410        4420        4430        4440
TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT

      4450        4460        4470        4480        4490        4500
TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG

      4510        4520        4530        4540        4550        4560
TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT

      4570        4580        4590        4600        4610        4620
CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC

      4630        4640        4650        4660        4670        4680
CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT

      4690        4700        4710        4720        4730        4740
ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG

      4750        4760        4770        4780        4790        4800
GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG

      4810        4820        4830        4840        4850        4860
CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC

      4870        4880        4890        4900        4910        4920
TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA

      4930        4940        4950        4960        4970        4980
ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG

      4990        5000        5010        5020        5030        5040
TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC

      5050        5060        5070        5080        5090        5100
ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA

      5110        5120        5130        5140        5150        5160
CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC
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      5170        5180        5190        5200        5210        5220
AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG

      5230        5240        5250        5260        5270        5280
TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC

      5290        5300        5310        5320        5330        5340
CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC

      5350        5360        5370        5380        5390        5400
AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG GCGACACGGA AATGTTGAAT

      5410        5420        5430        5440        5450        5460
ACTCATACTC TTCCTTTTTC AATATTATTG AAGCATTTAT CAGGGTTATT GTCTCATGAG

      5470        5480        5490        5500        5510        5520
CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC

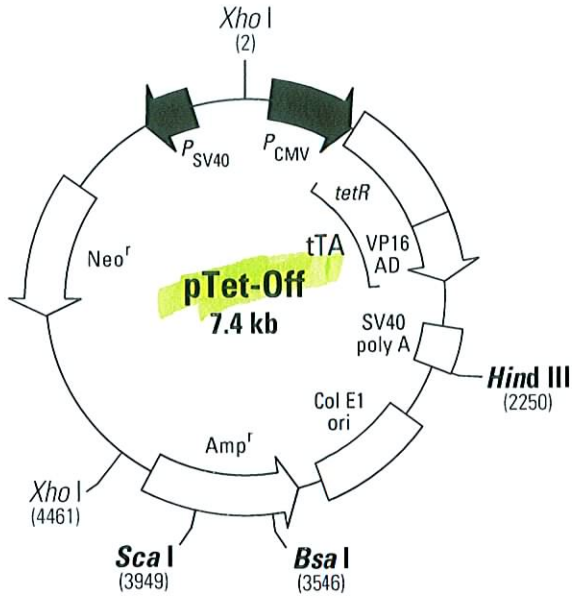
      5530        5540        5550        5560        5570        5580
CCGAAAAGTG CCACCTGACG TC..... ..... ..... .....
```

Vector Information

pTet-Off Vector Information

PT3073-5

Cat. No. 631017



Restriction Map of pTet-Off Vector. Unique restriction sites are in bold.

Description

pTet-Off expresses the tet-responsive transcriptional activator (tTA) from the strong immediate early promoter of cytomegalovirus (P_{CMV}). tTA is a fusion of amino acids 1–207 of the tet repressor (TetR) and the negatively charged C-terminal activation domain (130 amino acids) of the VP16 protein of herpes simplex virus. pTet-Off was originally described as pUHD15-1neo by Resnitzky *et al.* (1994) and was created by insertion of a neomycin resistance gene into pUHD15-1 (Gossen & Bujard, 1992). pTet-Off can be distinguished from pTet-On by digestion with *Hin*d III.

Use

The pTet-Off Vector is used to develop stable Tet-Off cell lines. After a vector that contains a gene under the control of a tet-responsive element (TRE) is transfected into a Tet-Off cell line, the tTA binds to the TRE, thus activating transcription in the absence of tetracycline (Tc), or its derivative doxycycline (Dox). As Tc or Dox is added to the culture medium, transcription from the TRE is turned off in a highly dose-dependent manner. More information on TRE-containing vectors and protocols describing the construction of Tet-Off cell lines can be found in the Tet Systems User Manual (PT3001-1).

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(PR23072; published 19 March 2002)

Location of Features

- Fragment containing P_{CMV} : 86–673
- Tetracycline-responsive transcriptional activator (tTA): 774–1781
- Col E1 origin of replication: 2604–3247
- Ampicillin resistance gene:
  β-lactamase coding sequences: 4255–3395
- Fragment containing the SV40 poly A signal: 1797–2254
- Neomycin/kanamycin resistance gene: 6462–5668
- SV40 promoter (P_{SV40}) controlling expression of neomycin/kanamycin resistance gene: 7125–6782.

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Tet Expression Systems and Cell Lines (July 1996) *Clontechniques* **XI**(3):2–5.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
4. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* **14**:1669–1679.

Note:

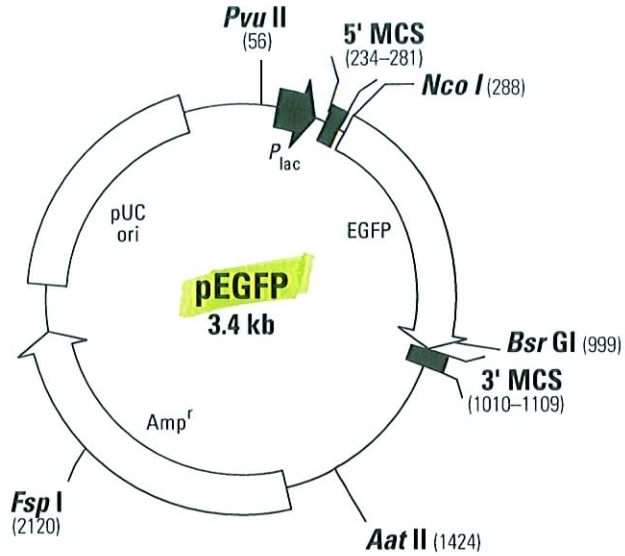
The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

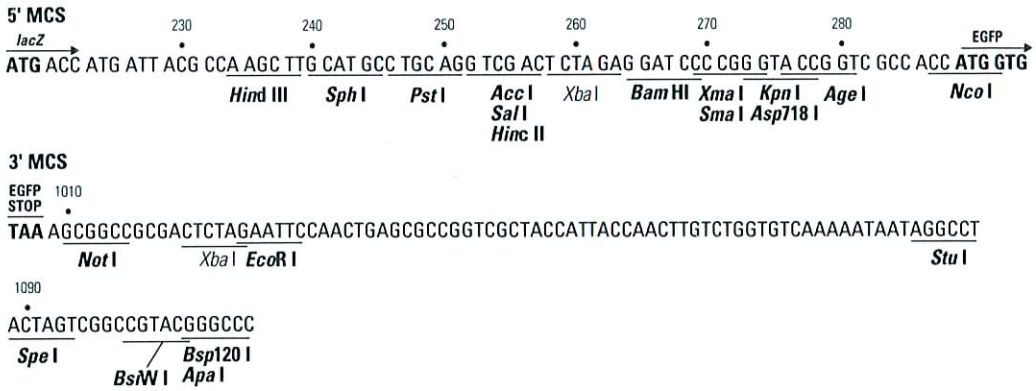
Vector Information

pEGFP Vector Information

PT3078-5

Catalog #6077-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP Vector. Unique restriction sites are in bold. The *Xba* I sites in the MCS can be used together to excise the EGFP gene.

Description:

pEGFP carries a red-shifted variant of wild-type green fluorescent protein (GFP) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP encodes the GFPmut1 variant (1) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (2). Upstream sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells.

The EGFP gene was cloned between the two MCS of the pUC19 derivative pPD16.43 (4). The EGFP coding sequence is flanked by separate MCS at the 5' and 3' ends, so the EGFP gene can be easily excised from pEGFP. Alternatively, the EGFP coding sequence can be amplified by PCR. The EGFP gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that a EGFP fusion protein is expressed from the *lac* promoter in *E. coli.* Note, however, that if you excise the EGFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) EGFP protein. The pUC backbone of EGFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli.*

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(PR29965; published 03 October 2002)

Location of features:

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*–EGFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon: 1006–1008
- 5' Multiple Cloning Site: 234–281
- Enhanced green fluorescent protein (EGFP) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 1006–1008
  - Insertion of Val at position 2: 292–294
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 481–486
  - His-231 to Leu mutation (A→T): 983
- 3' Multiple Cloning Site: 1010–1109
- Ampicillin resistance gene
  - Promoter: –35 region: 1485–1490; –10 region: 1508–1513
  - Transcription start point: 1520
  - Ribosome binding site: 1543–1547
  - β-lactamase coding sequences:
    - Start codon (ATG): 1555–1557; Stop codon: 2413–2415
    - β-lactamase signal peptide: 1555–1623
    - β-lactamase mature protein: 1624–2412
- pUC plasmid replication origin: 2563–3206

Primer location:

- EGFP-N Sequencing Primer (#6479-1): 355–334
- EGFP-C Sequencing Primer (#6478-1): 942–963

Propagation in *E. coli*:

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

invitrogen™

pcDNA™3.1(+)
pcDNA™3.1(–)

Catalog nos. V790-20 and V795-20

Version K
10 November 2010
28-0104

User Manual

Table of Contents

Important Information

pcDNA™ Vectors

This manual is supplied with the following products.

Product	Catalog no.
pcDNA™3.1(+) Vector	V790-20
pcDNA™3.1(–) Vector	V795-20

Shipping and Storage

Vectors are shipped on wet ice. Upon receipt, store at –20°C.

Contents

The pcDNA™3.1 vector components pcDNA™3.1 are listed below:

Item	Concentration	Volume
pcDNA™3.1 Vector pcDNA™3.1(+) **or** pcDNA™3.1(–)	20 µg at 0.5 µg/µl, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	40 µl
Control Plasmid pcDNA™3.1/CAT	20 µg at 0.5 µg/µl, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	40 µl

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Accessory Products

Additional Products

Additional products that may be used with the pcDNA™3.1 vectors are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10F' Chemically Competent Cells	20 reactions	C3030-03
	40 reactions	C3030-06
Lipofectamine™ 2000	1.5 ml	11668-019
	0.75 ml	11668-027
Geneticin®	1 g	11811-023
	5 g	11811-031
PureLink™ HQ Mini Plasmid Purification Kit	100 preps	K2100-01
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04

Methods

Overview

Description

pcDNA™3.1(+) and pcDNA™3.1(–) are 5.4 kb vectors derived from pcDNA™3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (–) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA™3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA™3.1.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50–100 µg/ml ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Cloning into pcDNA™3.1

Introduction

Diagrams are provided on pages 3-4 to help you design a cloning strategy for ligating your gene of interest into pcDNA™3.1. General considerations for cloning and transformation are listed below.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, please refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the propagation of this vector including TOP10F′, DH5α™-T1^R, and TOP10. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A-deficient (*end*A).

Note

If you wish to express a human gene of interest from pcDNA™3.1, we recommend using an Ultimate™ Human ORF (hORF) Clone available from Invitrogen. For more information about the Ultimate™ hORF Clones available, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 13).

Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintenance of pcDNA™3.1

To propagate and maintain pcDNA™3.1, use 10 ng of vector to transform a *rec*A, *end*A *E. coli* strain like TOP10F′, DH5α™-T1^R, TOP10, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin. Be sure to prepare a glycerol stock of your plasmid-containing *E. coli* strain for long-term storage (see page 5).

Cloning Considerations

pcDNA™3.1(+) and pcDNA™3.1(–) are non-fusion vectors. Your insert should contain a Kozak translation initiation sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

(G/A)NN<u>ATG</u>**G**

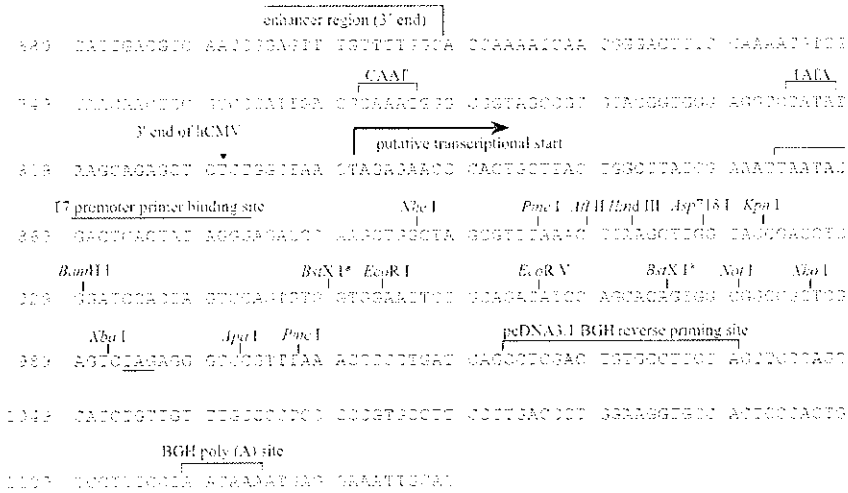
Your insert must also contain a stop codon for proper termination of your gene. Please note that the *Xba* I site contains an internal stop codon (TC<u>TAG</u>A).

continued on next page

Cloning into pcDNA™3.1, continued

Multiple Cloning Site of pcDNA™3.1(+)

Below is the multiple cloning site for pcDNA™3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™3.1(+) is available for downloading from our web site (www.invitrogen.com) or from Technical Support (see page 13).** For a map and a description of the features of pcDNA™3.1(+), please refer to the **Appendix**, pages 10-11.



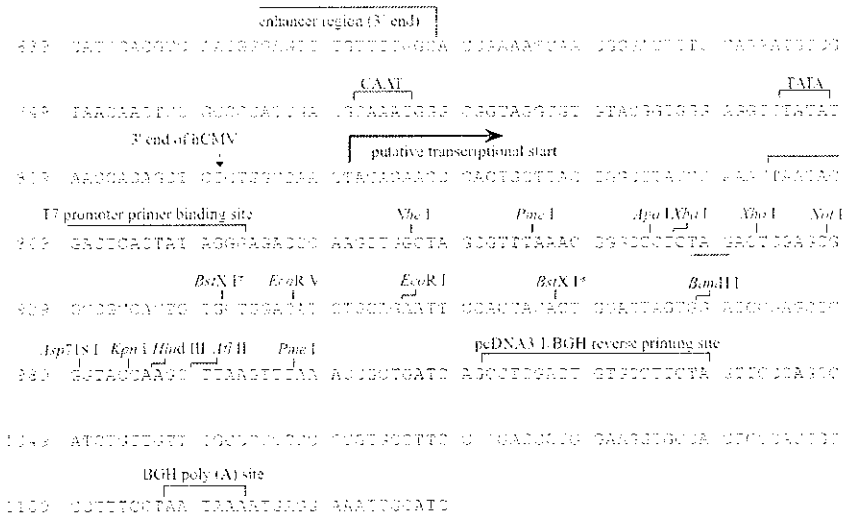
*Please note that there are two *BstX* I sites in the polylinker.

continued on next page

Cloning into pcDNA™3.1, continued

Multiple Cloning Site of pcDNA™3.1(–)

Below is the multiple cloning site for pcDNA™3.1(–). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA™3.1(–) is available for downloading from our web site (www.invitrogen.com) or from Technical Support (see page 13).** For a map and a description of the features of pcDNA™3.1(–), please see the **Appendix**, pages 10-11.

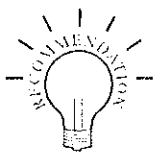


*Please note that there are two *Bst*X I sites in the polylinker.

Cloning into pcDNA™3.1, continued

E. coli Transformation

Once you have obtained a clone containing your gene of interest, you may transform the clone into a suitable *E. coli* host (see below). We recommend including a negative control in your experiment to help you evaluate your results.



We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 µg aliquots.

Preparing a Glycerol Stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at –20°C.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 ml of LB containing 50 µg/ml ampicillin.
- Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.

Store at –80°C.

continued on next page

Transfection

Introduction

Once you have verified that your gene is cloned in the correct orientation and contains an initiation ATG and a stop codon, you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results.

Plasmid Preparation

Once you have generated your expression clone, you must isolate plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be clean and free contamination with from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01), the PureLink™ HiPure Plasmid Midiprep Kit (Catalog no. K2100-04), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (*e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). For high efficiency transfection in a broad range of mammalian cell lines, we recommend using Lipofectamine™ 2000 Reagent (Catalog no. 11668-027) available from Invitrogen. For more information about Lipofectamine™ 2000 and other transfection reagents, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 13).

Positive Control

pcDNA™3.1/CAT is provided as a positive control vector for mammalian transfection and expression (see page 12) and may be used to optimize transfection conditions for your cell line. The gene encoding chloramphenicol acetyl transferase (CAT) is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in CAT expression that can be easily assayed (see below).

Assay for CAT Protein

You may assay for CAT expression by ELISA assay, western blot analysis, fluorometric assay, or radioactive assay (Ausubel *et al.*, 1994; Neumann *et al.*, 1987). If you wish to detect CAT protein using western blot analysis, you may use the Anti-CAT Antiserum (Catalog no. R902-25) available from Invitrogen. Other kits to assay for CAT protein using ELISA assay are available from Roche Molecular Biochemicals (Catalog no. 1 363 727) and Molecular Probes (Catalog no. F-2900).

continued on next page

Creating Stable Cell Lines

Introduction

The pcDNA™3.1(+) and pcDNA™3.1(–) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

To obtain stable transfectants, we recommend that you linearize your pcDNA™3.1 construct before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is not located within a critical element or within your gene of interest.

Geneticin®

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn5, results in detoxification of Geneticin® (Southern and Berg, 1982).

Determining Antibiotic Sensitivity

To successfully generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. Test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 6–7 plates. Add the following concentrations of antibiotic to each plate:
   - For Geneticin® selection, test 0, 50, 125, 250, 500, 750, and 1000 µg/ml Geneticin®.
2. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.
3. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth within 1–3 weeks after addition of the antibiotic.

Geneticin® Selection Guidelines

Once you have determined the appropriate Geneticin® concentration to use for selection, you can generate a stable cell line expressing your **pcDNA™3.1** construct. Geneticin® is available separately from Invitrogen (see page vi for ordering information). Use as follows:

1. Prepare Geneticin® in a buffered solution (*e.g.* 100 mM HEPES, pH 7.3).
2. Use the predetermined concentration of Geneticin® in complete medium.
3. Calculate concentration based on the amount of active drug.
4. Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 3 weeks of growth in selective medium.

continued on next page

Creating Stable Cell Lines, continued

Possible Sites for Linearization of pcDNA3.1(+)

Prior to transfection, we recommend that you linearize the pcDNA™3.1(+) vector. Linearizing pcDNA™3.1(+) will decrease the likelihood of the vector integrating into the genome in a way that disrupts the gene of interest or other elements required for expression in mammalian cells. The table below lists unique restriction sites that may be used to linearize your construct prior to transfection. **Other unique restriction sites are possible.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp)	Location	Supplier
Bgl II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
Mfe I	161	Upstream of CMV promoter	New England Biolabs
*Bst*1107 I	3236	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
*Eam*1105 I	4505	Ampicillin gene	AGS*, Fermentas, Takara
Pvu I	4875	Ampicillin gene	Invitrogen, Catalog no. 25420-019
Sca I	4985	Ampicillin gene	Invitrogen, Catalog no. 15436-017
Ssp I	5309	*bla* promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

Possible Sites for Linearization of pcDNA™3.1(–)

The table below lists unique restriction sites that may be used to linearize your pcDNA™3.1(–) construct prior to transfection. **Other unique restriction sites are possible.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp)	Location	Supplier
Bgl II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
Mfe I	161	Upstream of CMV promoter	New England Biolabs
*Bst*1107 I	3235	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
*Eam*1105 I	4504	Ampicillin gene	AGS*, Fermentas, Takara
Pvu I	4874	Ampicillin gene	Invitrogen, Catalog no. 25420-019
Sca I	4984	Ampicillin gene	Invitrogen, Catalog no. 15436-017
Ssp I	5308	*bla* promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

continued on next page

Creating Stable Cell Lines, continued

Selection of Stable Integrants

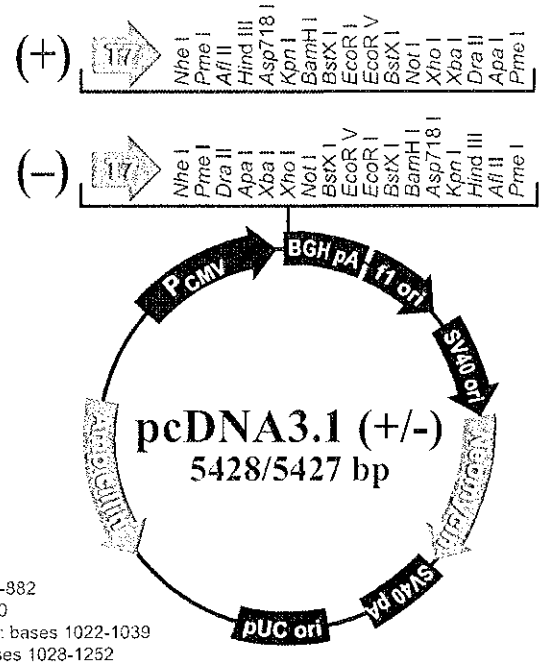
Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

1. Transfect your mammalian host cell line with your pcDNA™3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA™3.1/CAT plasmid as a positive control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the predetermined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
4. Feed the cells with selective medium every 3–4 days until Geneticin®-resistant foci can be identified.
5. Pick and expand colonies in 96- or 48-well plates.

Appendix
pcDNA™3.1 Vectors

Map

The figure below summarizes the features of the pcDNA™3.1(+) and pcDNA™3.1(–) vectors. **The complete sequences for pcDNA™3.1(+) and pcDNA™3.1(–) are available for down-loading from our World Wide Web site (www.invitrogen.com) or from Technical Support (see page 13).** Details of the multiple cloning sites are shown on page 3 for pcDNA™3.1(+) and page 4 for pcDNA™3.1(–).



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

continued on next page

pcDNA™3.1 Vectors, continued

Features

pcDNA™3.1(+) (5428 bp) and pcDNA™3.1(–) (5427 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/ enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in forward or reverse orientation	Allows insertion of your gene and facilitates cloning
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*
Ampicillin (*bla*) resistance gene (β-lactamase)	Allows selection of transformants in *E. coli*

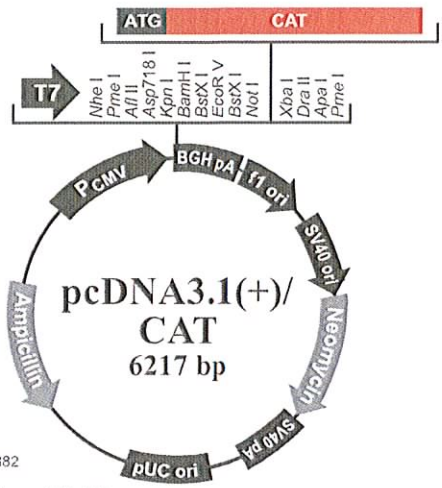
pcDNA™3.1/CAT

Description

pcDNA™3.1/CAT is a 6217 bp control vector containing the gene for CAT. It was constructed by digesting pcDNA™3.1(+) with *Xho* I and *Xba* I and treating with Klenow. An 800 bp *Hin*d III fragment containing the CAT gene was treated with Klenow and then ligated into pcDNA™3.1(+).

Map

The figure below summarizes the features of the pcDNA™3.1/CAT vector. **The complete nucleotide sequence for pcDNA™3.1/CAT is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Support (see page 13).**



Comments for pcDNA3.1(+)/CAT
6217 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
CAT ORF: bases 1027-1686
pcDNA3.1/BGH reverse priming site: bases 1811-1828
BGH polyadenylation sequence: bases 1817-2041
f1 origin: bases 2087-2515
SV40 early promoter and origin: bases 2520-2863
Neomycin resistance gene (ORF): bases 2925-3719
SV40 early polyadenylation sequence: bases 3893-4023
pUC origin: bases 4406-5076 (complementary strand)
Ampicillin resistance gene (ORF): bases 5221-6081 (complementary strand)

Technical Support

World Wide Web

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog

Additional product information and special offers

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For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

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The Certificate of Analysis (CofA) provides detailed quality control information for each product. CofAs are available on our website at www.invitrogen.com/support, and are searchable by product lot number, which is printed on each box.

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Introduction

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addgene

Find this plasmid at: www.addgene.org
Enter "25536" in the search box

Plasmid 25536: NME3

Gene/insert name:	NME3
Alternative names:	PDB: 1ZS6 Nucleoside-diphosphate Kinase 3 c371H6.2, DR-nm23, KIAA0516
Insert size (bp):	507
Gene/insert aliases:	NME3, NDPKC, NDPK-C, NM23H3, DR-nm23, NM23-H3, KIAA0516, c371H6.2
Species of gene(s):	H. sapiens (human)
Fusion proteins or tags:	His
Terminal:	N terminal on insert
Vector backbone:	pET28a-LIC (Search Vector Database)
Backbone manufacturer:	SGC
Type of vector:	Bacterial expression
Backbone size (bp):	7328
5' Sequencing primer:	T7 (List of Sequencing Primers)
3' Sequencing primer:	T7-term
Bacteria resistance:	Kanamycin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/25536
Plasmid Provided In:	DH5a
Principal Investigator:	Cheryl Arrowsmith

Comments: PDB: 1ZS6 http://www.thesgconline.org/SGC-WebPages/StructureDescription/1ZS6.php

Please acknowledge the principal investigator if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 25536" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/25536 for updated plasmid information and related links.

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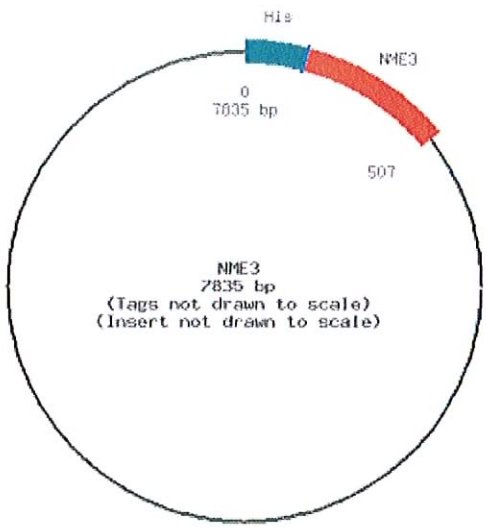
Find this plasmid at: www.addgene.org
Enter "25536" in the search box



Please check www.addgene.org/25536 for updated plasmid information and related links.

Page 2 of 2 - Date: 03/08/2011

MSDS and Description

There is no MSDS that I am aware of for the requested products, but the product sheets and plasmid maps are provided. The pET23b-Casp6-His, pCDNA3-CrmA, pCDNA3-Xiap-myc and pCDNA3-Bcl2 DNA plasmids are shipped as bacterial stabs in the E. coli strain DH5α or XL-1 Blue (both of which are approved microorganisms on our permit). These E. coli will be grown and the plasmid DNA isolated. pCDNA3-CrmA, pCDNA3-Xiap-myc and pCDNA3-Bcl2 DNA will be used for transfection of approved mammalian cell lines. Cell lysates will be analyzed using various biochemical methods. pET23b-Casp6-His DNA will be used to transform BL-21 E. coli cells (an approved microorganism on our permit). These cells will generate Casp6 protein which will be purified and used in biochemical assays.

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Find this plasmid at: www.addgene.org
Enter "11833" in the search box

Plasmid 11833: pcDNA3-Xiap-Myc

Gene/insert name:	XIAP
Insert size (bp):	1600
Gene/insert aliases:	XIAP, API3, ILP1, MIHA, XLP2, BIRC4
Species of gene(s):	H. sapiens (human)
Fusion proteins or tags:	Myc
Terminal:	N terminal on insert
Vector backbone:	pcDNA3.1 (Search Vector Database)
Type of vector:	Mammalian expression
Backbone size (bp):	5428
Cloning site 5':	Don't Know
Site destroyed during cloning:	Unknown
Cloning site 3':	Don't Know
Site destroyed during cloning:	Unknown
5' Sequencing primer:	T7 (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
Sequence:	Visit www.addgene.org/11833
Plasmid Provided In:	DH5a
Principal Investigator:	Guy Salvesen

Please acknowledge the principal investigator if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11833" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

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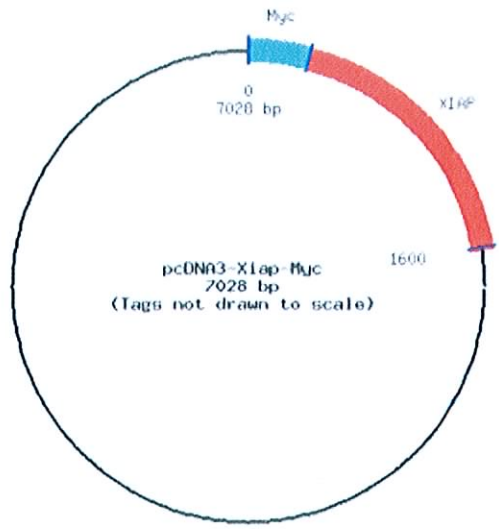


Please check www.addgene.org/11833 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "8768" in the search box

Plasmid 8768: 3336 pcDNA3 Bcl-2

Gene/insert name:	Bcl-2
Insert size (bp):	720
GenBank/Entrez ID of insert:	NM_000633
Gene/insert aliases:	BCL2, Bcl-2
Species of gene(s):	H. sapiens (human)
Vector backbone:	pcDNA3 (Search Vector Database)
Backbone manufacturer:	Invitrogen
Type of vector:	Mammalian expression
Backbone size (bp):	5400
Cloning site 5':	EcoRI
Site destroyed during cloning:	Yes
Cloning site 3':	EcoRI
Site destroyed during cloning:	Yes
5' Sequencing primer:	CMV-F (List of Sequencing Primers)
3' Sequencing primer:	BGH-rev
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
Sequence:	Visit www.addgene.org/8768
Author's Map:	Visit www.addgene.org/8768
Plasmid Provided In:	XL1 Blue
Principal Investigator:	Stanley Korsmeyer

Comments: ORF contains SacI, PstI, and BamHI sites sense from T7 direction.

Article: BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Yamamoto K et al. (Mol Cell Biol 1999 Dec;19(12):8469-78. Pubmed)

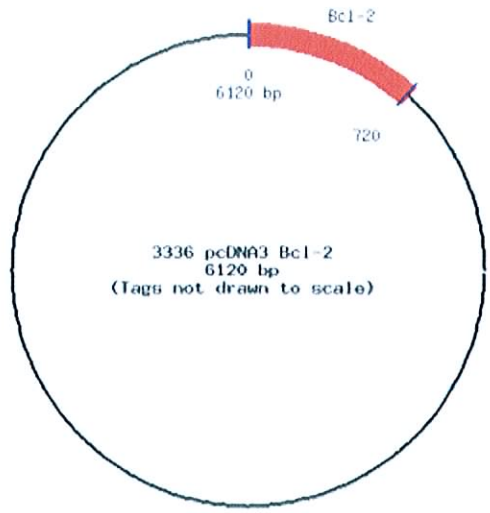
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 8768" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/8768 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "8768" in the search box



Please check www.addgene.org/8768 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "11832" in the search box

Plasmid 11832: pcDNA3-CrmA

Gene/insert name:	CrmA
Insert size (bp):	1050
Gene/insert aliases:	CrmA, CPXV207
Species of gene(s):	Cowpox virus
Vector backbone:	pcDNA3 (Search Vector Database)
Backbone manufacturer:	Invitrogen
Type of vector:	Mammalian expression
Backbone size (bp):	5446
Cloning site 5':	HindIII
Site destroyed during cloning:	Unknown
Cloning site 3':	XhoI
Site destroyed during cloning:	Unknown
5' Sequencing primer:	T7 (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
Sequence:	Visit www.addgene.org/11832
Plasmid Provided In:	DH5a
Principal Investigator:	Guy Salvesen

Article: FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogens. Muzio M et al. (J Biol Chem. 1997 Jan 31. 272(5):2952-6. Pubmed)

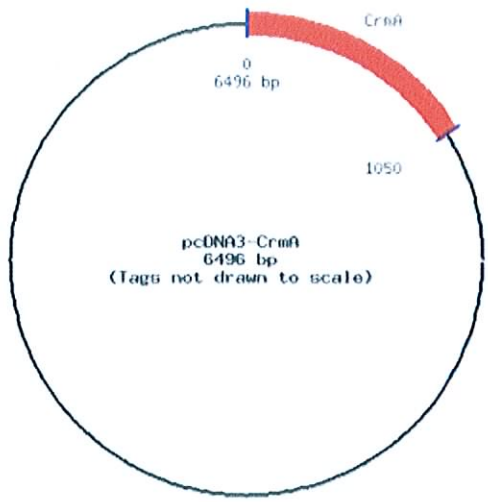
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11832" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11832 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "11832" in the search box



Please check www.addgene.org/11832 for updated plasmid information and related links.

Page 2 of 2 - Date: 05/30/2011

addgene

Find this plasmid at: www.addgene.org
Enter "11823" in the search box

Plasmid 11823: pET23b-Casp6-His

Gene/insert name:	Caspase 6
Insert size (bp):	988
Gene/insert aliases:	CASP6, MCH2
Species of gene(s):	H. sapiens (human)
Fusion proteins or tags:	6xHis
Terminal:	C terminal on insert
Vector backbone:	pET-23 b (Search Vector Database)
Type of vector:	Bacterial expression
Backbone size (bp):	3666
Cloning site 5':	XhoI
Site destroyed during cloning:	Unknown
Cloning site 3':	NdeI
Site destroyed during cloning:	Unknown
5' Sequencing primer:	T7 terminal primer (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/11823
Plasmid Provided In:	DH5a
Principal Investigator:	Guy Salvesen

Article: Target protease specificity of the viral serpin CrmA. Analysis of five caspases. Zhou Q et al. (J Biol Chem. 1997 Mar 21. 272(12):7797-800. Pubmed)

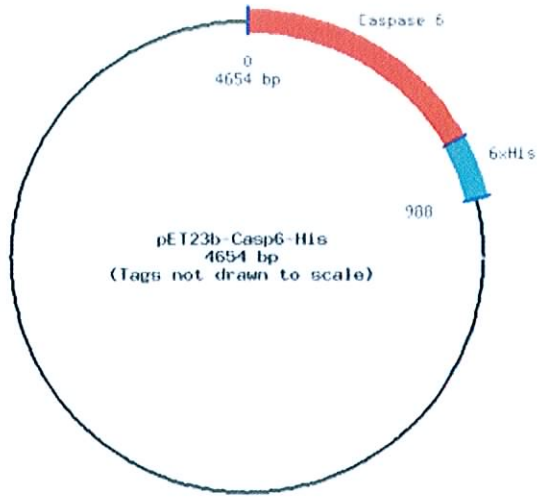
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11823" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11823 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "11823" in the search box



Please check www.addgene.org/11823 for updated plasmid information and related links.

Material Safety Data Sheet

Version 4.3
Revision Date 01/18/2011
Print Date 02/25/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Okadaic acid, from *Prorocentrum concavum*
Product Number	:	O9381
Brand	:	Sigma
Product Use	:	For laboratory research purposes.

Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer	: Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+19058299500		
Fax	:	+19058299292		
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

Toxin Info

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Smooth muscle.

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and	Highly Toxic
D2B	Serious Toxic Effects	Moderate skin irritant

GHS Classification

Acute toxicity, Oral (Category 3)
Acute toxicity, Inhalation (Category 3)
Acute toxicity, Dermal (Category 3)
Skin irritation (Category 2)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H301 + H311	Toxic if swallowed or in contact with skin.
H315	Causes skin irritation.
H331	Toxic if inhaled.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P280	Wear protective gloves/ protective clothing.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P311	Call a POISON CENTER or doctor/ physician.

HMIS Classification

Health hazard: 2

Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation Toxic if inhaled. Causes respiratory tract irritation.
Skin Toxic if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion Toxic if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : OA

Formula : $C_{44}H_{68}O_{13}$ $C_{44}H_{68}O_{13}$
Molecular Weight : 805.00 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Okadaic acid			
78111-17-8	-	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

Keep in a dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	white

Safety data

pH	no data available
Melting/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition	no data available

temperature	
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
TDLo Oral - mouse - 0.15 mg/kg

Inhalation LC50
Dermal LD50
Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

Genotoxicity in vitro - Human - lymphocyte
Sister chromatid exchange

Genotoxicity in vitro - Hamster - Lungs
Mutation in mammalian somatic cells.

Carcinogenicity

Carcinogenicity - mouse - Skin
Tumorigenic:Equivocal tumorigenic agent by RTECS criteria. Skin and Appendages: Other: Tumors.

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	Toxic if inhaled. Causes respiratory tract irritation.
Ingestion	Toxic if swallowed.
Skin	Toxic if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Signs and Symptoms of Exposure
Tumor promoter., Gastrointestinal disturbance

Synergistic effects
no data available

Additional Information
RTECS: AA8227800

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Okadaic acid)
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 3462 Class: 6.1 Packing group: I EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Okadaic acid)
Marine pollutant: No

IATA
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Okadaic acid)

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Okadaic acid	78111-17-8

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and	Highly Toxic
D2B	Serious Toxic Effects	Moderate skin irritant

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Okadaic Acid
Proposed Use Dose:	5 µg
Proposed Storage Dose:	100 µg
LD_{50} (species):	150 µg

Calculation:

150 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 7500

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 750

Comments/Recommendations: