

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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 a and Safety for a modification form. See website: www.uwo.ca

Level 3

Please ensure that all questions are fully and clearly answered and returned, which will cause delays in your approval and frustration.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	Stephen Barr
DEPARTMENT:	Microbiology & Immunology
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EMERGENCY PHONE NUMBER(S):	519-495-1490
EMAIL:	stephen.barr@uwo.ca

Location of experimental work to be carried out :

Building : Dental Sciences	Room(s): 6006
Building : _____	Room(s): _____
Building : _____	Room(s): _____

***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: **Currently unfunded**

GRANT TITLE(S): **Submitted (abbreviated titles): 1) UWO-ADF Small Grant: Impact of TRIM22 in HIV infection. 2) CIHR: Role of Herc5 in HIV infection.**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Sherry Xu	lxu48@uwo.ca	_____
Matthew Woods	mwoods22@uwo.ca	_____
Jenna Kelly	jkelly25@uwo.ca	_____
Clayton Hattlmann	chattlma@uwo.ca	_____
Macon Coleman	mcolem4@uwo.ca	_____

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

BACTERIAL WORK:

>>>Plasmid preparation- "Cloning strains" (eg. Escherichia coli DH5alpha) are used to propagate our plasmids for purification. Equipment is decontaminated with 10% bleach and washed with soapy water, and/or autoclaved.

MAMMALIAN WORK:

>>>Primary cells and cell lines- A variety of mammalian primary cells and cell lines will be cultured in our Level 2+3 room (DSB3006b2) and then transported up to our Level 3 room (DSB6006) in an approved leak-proof container. Cells are maintained in a CO2 incubator with daily sub-culturing for propagation. Cells are manipulated using various transfection techniques (eg. Lipofectamine) in a certified biological safety cabinet. Equipment/supplies/liquid in contact with the cells and/or virus are decontaminated with Wescodyne, autoclaved and incinerated or, in the case of liquid, poured down the sink with plenty of water.

VIRUS WORK:

Replication-competent lentiviruses/retroviruses will be produced by independently transfecting various mammalian cells with plasmids encoding full-length HIV-1 (human), HTLV-1 (human), SIV (simian) or EIAV (equine) provirus. All work will be carried out with Level 3 precautions in DSB6009 according to the SOPs. Virus produced from these cells will be stored frozen at -80C or used to infect susceptible cells. Infected cells will be fixed at a final concentration of 2% formaldehyde and subjected to Western blotting, confocal microscopy analysis or flow cytometry under Containment Level 1 conditions. Equipment/supplies/liquid in contact with the cells and/or virus are decontaminated with Wescodyne, autoclaved and incinerated or, in the case of liquid, poured down the sink with plenty of water. All biological samples leaving the Level 3 are fixed to a final concentration of 2% formaldehyde and are non-infectious. All transport vessels containing samples for processing under Level 1 conditions are transported to and from the Level 3 facility in approved leak-proof containers and all vessels (including tubes) are sprayed liberally with 70% ethanol.

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

UWO- ADF Small grant application: TRIM22 is a newly discovered protein in humans that blocks infection by the human immunodeficiency virus (HIV). TRIM22 targets two different parts of the HIV lifecycle by two different ways. One way involves blocking the production of HIV proteins within cells, and the other way prevents the assembly of the virus within cells. The Barr laboratory has discovered that specific mutations in the human TRIM22 gene can inactivate TRIM22. The objective of the proposed project is to determine if genetic differences in the TRIM22 gene can influence HIV infection in patients and their disease progression. The method of investigation will involve reading the genetic code of the TRIM22 gene from a variety of HIV-infected patients at different stages of disease progression. We will then determine if specific genetic differences are associated with protection from, or susceptibility to, HIV infection. Results from the proposed project could provide information for the development of drugs or gene therapy that can enhance the ability of TRIM22 to attack HIV. Our results could also identify a factor for personalized medicine that may help improve patient drug-treatment plans and help predict disease progression in infected individuals.

CIHR: HERC5 is a newly discovered antiviral protein that exhibits potent inhibitory properties towards HIV. The Barr laboratory focuses on the characterization of the molecular mechanism underlying its antiviral activities. Our research involves over-expressing and knocking down HERC5 expression levels in mammalian cells and assessing its effects on mammalian cells and on the ability of HIV to infect these cells (carried out under Level 3 conditions- see Barf UWO-BIO-00224). Our research involves determining how HERC5 inhibits HIV protein synthesis and assembly, and determining the impact of HERC5 gene polymorphisms on HIV infection. We are addressing three specific hypotheses: 1) HERC5 possesses guanine exchange factor activity that disrupts the nuclear export of RNA and hence synthesis of HIV protein; 2) HERC5 conjugates a small protein called ISG15 to target proteins that ultimately interferes with HIV assembly' 3) HERC5 gene polymorphisms exist in the human population to impact a person's susceptibility to HIV infection and disease progression to AIDS. We use a variety of gene transfection and gene transduction (pseudotyped HIV virus) techniques to express our HERC5 constructs in cells. HERC5 or the HERC5 mutant constructs generated in our laboratory are not known to exhibit oncogenic properties. We also perform biochemical analyses on recombinant HERC5 protein to identify and characterize its biological activity. This activity assay is not biohazardous. We will also isolate genomic DNA from healthy and HIV-infected patients in order to sequence the HERC5 gene and identify polymorphisms that may affect HERC5 function. Biosafety level 3 precautions are followed when handling HIV-infected samples (see Barf UWO-BIO-00224).

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES 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? YES 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:

Full Scientific 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
<i>Human Immunodeficiency Pseudovirus</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	0.1	NIH AIDS Reagents	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input checked="" type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Escherichia coli DH5alpha</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	Agilent Technologies	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Escherichia coli BL21(DE3)pLysS</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	Agilent Technologies	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Escherichia coli HB101</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	BioRad	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Escherichia coli Stbl4</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	1	Invitrogen	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link: http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf*

Additional Comments: _____

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES 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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Human patients	Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	See next page		
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Non-human primate	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	See next page		
Other (specify)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	EML-3C (equine)	1	ATCC

**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 1 2 2+ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES 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 other Body Fluid	Healthy donors	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid	PBMCs from HIV-infected donors	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> Unknown	HIV-1	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input checked="" type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

Cell Type	Is this cell type used in your work?	Specific cell lines	Containment Level	Viral components	Supplier
Human	Yes	293T	2	Adeno and SV-40 viral sequences	ATCC
	Yes	HOS	1		NIH AIDS Reagents
	Yes	GHOST(3)R3-X4-R5	1		NIH AIDS Reagents
	Yes	HeLa	2	Human Papilloma Virus	ATCC
	Yes	U2OS	1		ATCC
	Yes	143B	1		ATCC
	Yes	Jurkat E6-1	1		ATCC
	Yes	Supt1	1		NIH AIDS Reagents
	Yes	293T Phoenix	2+	Replication-defective HIV-1 packaging genes	S. Kim Lab (UWO)
	Yes	HUT78	1		NIH AIDS Reagents
	Yes	SKSM2	2+	Replication-defective HIV-1	R. Bushman Lab (Upenn)
	Yes	U38-Cat	1		NIH AIDS Reagents
	Yes	CEM-SS	1		NIH AIDS Reagents
	Yes	CEM-GFP	1		NIH AIDS Reagents
Yes	CEM-T4	1		NIH AIDS Reagents	
Yes	H9	1		NIH AIDS Reagents	
Yes	HeLaCD4-Cat	2	Human Papilloma Virus	NIH AIDS Reagents	
Yes	Hs 181.Tes	1		ATCC	
Non-human Primate	Yes	COS-1	2+	Papovavirus	R. Bushman Lab (Upenn)
	Yes	COS-7	2+	SV40 viral sequences	R. Bushman Lab (Upenn)
	Yes	Vero	2+		J. Smiley Lab (U Alberta)
	Yes	Macaca T cell line	2+		NHPRR
Murine	Yes	TM4	1		ATCC
Equine	Yes	EML-3C	1		ATCC

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? YES NO If **NO**, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
Escherichia coli: (DH5alpha, HB101, or Stbl4)	See attached page					

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

** Please attach a plasmid map.

***No Material Safety Data Sheet is required for the following strains of *E. coli*:

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
HIV	pR9	R. Bushman (Upenn)	HIV genome	Cells will produce HIV.
SIV	pSIVgorCP2139	AIDS Reagents	SIV genome	Cells will produce SIV.
EIAV	pSIVagmTan-1 pEIAV	AIDS Reagents AIDS Reagents	SIV genome EIAV genome	Cells will produce EIAV.
HTLV-1	pMT-2.HTLV-1 pHTLV1-K30	AIDS Reagents AIDS Reagents	HTLV-1 genome HTLV-1 genome	Cells will produce HTLV-1; oncogenic.

* Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? YES NO

4.3.2 Will virus be infectious to humans or animals? YES NO

4.3.3 Will this be expected to increase the containment level required? YES NO

5.0 Will genetic sequences from the following be involved?

- ◆ HIV NO YES, specify **Full genome**
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens NO YES, specify **HTLV-1**
- ◆ SV 40 Large T antigen NO YES
- ◆ E1A oncogene NO YES
- ◆ Known oncogenes NO YES, specify **HTLV-1**
- ◆ Other human or animal pathogen and or their toxins NO YES, specify **SIV, EIAV**

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: **HTLV= human T-lymphotropic virus; SIV= Simian immunodeficiency virus;**
EIAV= equine infectious anemia virus;

Plasmids	Plasmid Source	Gene transformed/transfected	Change due to transformation of bacteria?	Change in pathogenicity of bacteria after modification?	Consequences due to transformation of bacteria?
pCDNA3.1	Invitrogen	TRIM22, HERC1-6	None known	None	Plasmid propagation
pCS2	Clontech	HERC1-6	None known	None	Plasmid propagation
pLKO.1	Open Biosystems	shRNA (TRIM22, HERC1-6, eGFP, scrambled)	None known	None	Plasmid propagation
pdeltaR9	R. Bushman (Upenn)	HIV genome minus envelope and Nef	None known	None	Plasmid propagation
pVSVG	R. Bushman (Upenn)	Vesicular stomatitis virus protein G	None known	None	Plasmid propagation
pPPT	R. Bushman (Upenn)	GFP	None known	None	Plasmid propagation
pGag	NIH AIDS Reagents	HIV Gag	None known	None	Plasmid propagation
pEnv	NIH AIDS Reagents	HIV Env	None known	None	Plasmid propagation
pRev	NIH AIDS Reagents	HIV Rev	None known	None	Plasmid propagation
pTat	NIH AIDS Reagents	HIV Tat	None known	None	Plasmid propagation
pNef	NIH AIDS Reagents	HIV Nef	None known	None	Plasmid propagation
pVpu	NIH AIDS Reagents	HIV Vpu	None known	None	Plasmid propagation
pVpr	NIH AIDS Reagents	HIV Vpr	None known	None	Plasmid propagation
p3xFLAG-CMV-10	Sigma	TRIM22, HERC1-6	None known	None	Plasmid propagation
pTRE2hyg-HIV protease	Clontech	HIV protease	None known	None	Plasmid propagation
pLPCX	Clontech	TRIM22	None known	None	Plasmid propagation
pLRCX	Clontech	TRIM22	None known	None	Plasmid propagation
pSIVgorCP2139	NIH AIDS Reagents	SIV genome	None known	None	Plasmid propagation
pSIVagmTan-1	NIH AIDS Reagents	SIV genome	None known	None	Plasmid propagation
pC15CAT	NIH AIDS Reagents	chloramphenicol acetyltransferase	None known	None	Plasmid propagation
pMT-2-HTLV1	G. Dekaban (UWO)	HTLV-1 genome	None known	None	Plasmid propagation
pHTLV1-K30	NIH AIDS Reagents	HTLV-1 genome	None known	None	Plasmid propagation
pTeton	Clontech	TRIM22, HERC1-6	None known	None	Plasmid propagation
pUbiquitin(HA tagged)	K. Chin (Genome Institute of Singapore)	ubiquitin	None known	None	Plasmid propagation
pUbiquitin(His tagged)	K. Chin (Genome Institute of Singapore)	ubiquitin	None known	None	Plasmid propagation
pISG15	K. Chin (Genome Institute of Singapore)	interferon stimulated gene 15	None known	None	Plasmid propagation
pUbe1L	K. Chin (Genome Institute of Singapore)	E1 activating enzyme for ISG15	None known	None	Plasmid propagation
pUbch8	K. Chin (Genome Institute of Singapore)	E2 activating enzyme for ISG15	None known	None	Plasmid propagation
pUbp43	Open Biosystems	ISG15 deconjugase	None known	None	Plasmid propagation
pGL3	Promega	None-Empty vector control	None known	None	Plasmid propagation

pMLVgag	R. Bushman (Upenn)	MLV Gag Pol	None known	None	Plasmid propagation
Plasmids	Plasmid Source	Gene transformed/transfected	Change due to transformation of bacteria?	Change in pathogenicity of bacteria after modification?	Consequences due to transformation of bacteria?
pEIAV	NIH AIDS Reagents	EIAV genome	None known	None	Plasmid propagation
pEBFP	Clontech	Blue Fluorescent protein	None known	None	Plasmid propagation
pECFP	Clontech	Cyan fluorescent protein	None known	None	Plasmid propagation
pEGFP-C1	Clontech	Green fluorescent protein	None known	None	Plasmid propagation
pEGFP-N3	Clontech	Green fluorescent protein	None known	None	Plasmid propagation
pET28a	Novagen	TRIM22, HERC5	None known	None	Plasmid propagation
pET41a	Novagen	TRIM22, HERC5	None known	None	Plasmid propagation
pEYFP-N1	Clontech	Yellow fluorescent protein	None known	None	Plasmid propagation

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? YES, number: NO PENDING

7.0 Animal Experiments

7.1 Will live animals be used? YES NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

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

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? YES NO

8.3 If **YES**, please specify the animal(s) used:

- | | | |
|-----------------------------|--|-----------------------------|
| ◆ Pound source dogs | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Pound source cats | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Cattle, sheep or goats | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Non-human primates | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Wild caught animals | <input type="checkbox"/> YES, species & colony # | <input type="checkbox"/> NO |
| ◆ Birds | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify | <input type="checkbox"/> NO |

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? YES NO

If **YES**, Please provide details:

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? YES 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? Initiate and maintain colony, give location:

"One-time" use, give location:

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

10.7 Do you use insects that require a permit from the CFIA permit? YES NO

If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

- 11.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # **PHAC:P-17116; CFIA:A-2009-0355** NO

13.0 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.

An X in the check box indicates you agree with the above statement...
Enter Your Name Stephen Barr **Date:** November 1, 2011

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
 YES, location and date of most recent biosafety inspection: **October 2010**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-00224**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? YES NO
If **YES** please describe:

15.2 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:
SOPs will be followed. Injured area will be scrubbed with soapy water, rinsed with flowing water. Person will go to Staff Health during work hours or to the emergency room after hours.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html>

An X in the check box indicates you agree with the above statement...
Enter Your Name Stephen Barr **Date:** November 1, 2011

15.4 Additional Comments: _____

16.0 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:



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 considered to be level 1 animal pathogens:

- | | | | | |
|---------------|--------------------|-----------|-------------------|----------------|
| • 5K | • CIE85 | • J52 | • MC4100 (MuLac) | • U5/41 |
| • 58 | • DH1 | • J53 | • MG1655 | • W208 |
| • 58-161 | • DH10 GOLD | • JC3272 | • MM294 | • W945 |
| • 679 | • DH10B | • JC7661 | • MS101 | • W1485 |
| • 1532 | • DH5 | • JC9387 | • NC-7 | • W3104 |
| • AB284 | • DH5-alpha | • JF1504 | • Nissle 1917 | • W3110 |
| • AB311 | • DP50 | • JF1508 | • One Shot STBL3 | • WA704 |
| • AB1157 | • DY145 | • JF1509 | • OP50 | • WP2 |
| • AB1206 | • DY380 | • JJ055 | • P678 | • X1854 |
| • AG1 | • E11 | • JM83 | • PA309 | • X2160T |
| • B | • EJ183 | • JM101 | • PK-5 | • X2541 |
| • BB4 | • EL250 | • JM109 | • PMC103 | • X2547T |
| • BD792 | • EMG2 | • K12 | • PR13 | • XL1-BLUE |
| • BL21 | • EPI 300 | • KC8 | • Rri | • XL1-BLUE-MRF |
| • BL21 (DE3) | • EZ10 | • KA802 | • RV308 | • XL0LR |
| • BM25.8 | • FDA Seattle 1946 | • KAM32 | • S17-1λ -PIR | • Y10 |
| • C | • Fusion-Blue | • KAM33 | • SCS1 | • Y1090 (1090) |
| • C-1a | • H1443 | • KAM43 | • SMR10 | • YN2980 |
| • C-3000 | • HF4714 | • LE450 | • SOLR | • W3110 |
| • C25 | • HB101 | • LE451 | • SuperchargeEZ10 | • WG1 |
| • C41 (DE3) | • HS(PFAMP)R | • LE452 | • SURE | • WG439 |
| • C43 (DE3) | • Hfr3000 | • MB408 | • TOP10 | • WG443 |
| • C600 | • Hfr3000 X74 | • MBX1928 | • TG1 | • WG445 |
| • Cavalli Hfr | • HMS174 | • MC1061 | | |

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

MSDS for SIV

MSDS for SIV is not available.

SIV belongs to the Family *Retroviridae* and Genus *Lentivirus* as is HIV. **Simian immunodeficiency virus (SIV)** is a [retrovirus](#) that is found, in numerous strains, in [primates](#); the specific strains infecting [humans](#) are [HIV-1](#) and [HIV-2](#), the viruses that cause [AIDS](#). Therefore, similar precautions should be taken as with HIV.

MSDS for Recombinant Lentiviral Vector (HIV-based):

There is no specific MSDS for this vector. This vector is based on HIV components except that the virus generated is replication incompetent. Therefore, the MSDS will be similar to that of HIV (see Section 1.2). This vector is used for gene delivery into target cells.

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)

Chemtrec: (800) 424-9300

To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I**Hazardous Ingredients**

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II**Physical data**

Pink or red aqueous liquid

SECTION III**Health hazards****For Biosafety Level 1 Cell Lines**

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV**Fire and explosion**

Not applicable

SECTION V**Reactivity data**

Stable. Hazardous polymerization will not occur.

SECTION VI**Method of disposal**

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII**Special protection information****For Biosafety Level 1 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII**Special precautions or comments**

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the *Journal of Tissue Culture Methods* (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at

<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

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February 2002

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Agency of CanadaAgence de la santé
publique du Canada

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Home : Material Safety Data Sheets - Infectious Substances :

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES**SECTION I - INFECTIOUS AGENT****NAME:** *Human Immunodeficiency Virus***SYNONYM OR CROSS REFERENCE:** HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV**CHARACTERISTICS:** Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase**SECTION II - HEALTH HAZARD****PATHOGENICITY:** Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency**EPIDEMIOLOGY:** First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category**HOST RANGE:** Humans**INFECTIOUS DOSE:** Unknown**MODE OF TRANSMISSION:** Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur**INCUBATION PERIOD:** Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years**COMMUNICABILITY:** Period of communicability extends from asymptomatic period through appearance of opportunistic diseases**SECTION III - DISSEMINATION****RESERVOIR:** Humans**ZOONOSIS:** None**VECTORS:** None**SECTION IV - VIABILITY****DRUG SUSCEPTIBILITY:** Several reverse transcriptase and protease inhibitors now licensed**SUSCEPTIBILITY TO DISINFECTANTS:** Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol**PHYSICAL INACTIVATION:** Effectiveness of 56°C - 60°C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56°C before serologic testing reduces residual infectivity to below detectable levels**SURVIVAL OUTSIDE HOST:** Drying in environment causes rapid (within several hours) 90-99% reduction in HIV concentration**SECTION V - MEDICAL****SURVEILLANCE:** Serological monitoring for evidence of HIV infection**FIRST AID/TREATMENT:** Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV**IMMUNIZATION:** None available**PROPHYLAXIS:** Experimental prophylaxis with AZT/DDI or other appropriate drug**SECTION VI - LABORATORY HAZARDS****LABORATORY-ACQUIRED INFECTIONS:** 5 reported laboratory acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwideOffice of
Laboratory
Security

MSDS

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products

PRIMARY HAZARDS: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and splashing infected materials - virus should be presumed in/on all equipment and devices coming in direct contact with infected materials

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and non-cultured procedures (primary containment devices may be indicated eg. biological safety cabinets) and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV; Biosafety level 3 practices, containment equipment and facilities for all work culturing HIV

PROTECTIVE CLOTHING: Gloves should be worn when handling potentially infectious specimens, cultures or tissues; laboratory coats, gowns or suitable protective clothing should be worn

OTHER PRECAUTIONS: Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal - steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: September 1996 **Prepared by:** Office of Biosafety

LCDC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

[\[Material Safety Data Sheets - Index\]](#)

Last Updated: 1997-10-11



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Info on Cell Line(s)

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Product Description

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Cell Biology

ATCC® Number: **CRL-8303™** Order this Item

Price: **\$329.00**

Designations: **143B**

Depositors: Wistar Institute

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: mixed

Source: **Organ:** bone
Disease: osteosarcoma

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

DNA Profile (STR): Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 10,13
D5S818: 13
D7S820: 11,12
THO1: 6
TPOX: 11
vWA: 18

Age: 13 year old

Gender: female

Ethnicity: Caucasian

Comments: Thymidine kinase negative (TK-).
This is a human osteosarcoma cell line.

Propagation: **ATCC complete growth medium:** Minimum essential medium (Eagle) in Earle's BSS with 0.015 mg/ml 5-bromo-2'-deoxyuridine, 90%; fetal bovine serum, 10%
Temperature: 37.0°C

Subculturing: **Medium Renewal:** 2 to 3 times per week
Remove medium, rinse with fresh 0.25% trypsin, 0.02% EDTA solution and allow the cells to sit at room temperature (or at 37C) until they detach (about 10 minutes). Add fresh medium, aspirate and dispense into new flasks.

Related Links



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[Related Cell Culture Products](#)

Preservation: 90% FBS; 10% DMSO

Related Products: derivative:ATCC CRL-8304

References:

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

32519: Roller RJ, et al. Structure and function in the herpes simplex virus 1 RNA-binding protein US11: mapping of the domain required for ribosomal and nucleolar association and RNA binding in vitro. *J. Virol.* 70: 2842-2851, 1996. PubMed: 8627758

33047: Hofhaus G, et al. Respiration and growth defects in transmtochondrial cell lines carrying the 11778 mutation associated with Leber's hereditary optic neuropathy. *J. Biol. Chem.* 271: 13155-13161, 1996. PubMed: 8662757

33152: Hocking AM, et al. Eukaryotic expression of recombinant biglycan. *J. Biol. Chem.* 271: 19571-19577, 1996. PubMed: 8702651

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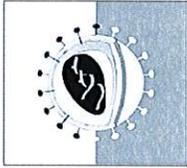
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NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
Bldg. 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686-4740
Fax: 301-515-4015
www.aidsreagent.org

DATA SHEET

Reagent: U38

Catalog Number: 1297

Lot Number: 2 95082

Provided: 6.3 x 10⁶ cells/vial.

Propagation Medium: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze Medium: RPMI 1640, 70%; fetal bovine serum, 20%; DMSO, 10%.

Growth Characteristics: Split twice weekly 1:10. U38 cells are stable and do not need to be maintained in selection medium. If growth in selection medium is desired, propagation medium containing 700 µg/ml G418 should be used. Wash the thawed cells in propagation medium and centrifuge for 10 minutes at 1000 rpm before seeding the cells in a culture flask.

Sterility: Negative for bacteria, fungi, and mycoplasma.

Special Characteristics: U38¹ is a U937 derivative that contains stably integrated, silent copies of the HIV-1 LTR promoter linked to the CAT gene. This cell line was generated by infection of U937 cells with a helper-free recombinant retroviral vector containing the HIV-1 LTR-CAT gene construct. U38 was selected in geneticin (G418) under limiting dilution and is a sensitive indicator cell line for HIV-1 Tat. When infected by HIV-1, U38 produces high levels of chloramphenicol acetyl transferase (CAT)^{1,2}. Morphology is monocyte-like. Contains LTR sequences to +80 in the R region. Contains the entire U3 region, but lacks U5 sequences.

Recommended Storage: Liquid nitrogen.

Contributor: Dr. Barbara K. Felber and Dr. George N. Pavlakis.

References: ¹Felber BK, Pavlakis GN. A quantitative bioassay for HIV-1 based on trans-activation. *Science* **239**:184-187, 1988.
²Schwartz S, Felber BK, Fenyo EM, Pavlakis GN. Rapidly and slowly replicating human immunodeficiency virus type 1 isolates can be distinguished according to target-cell tropism in T-cell and monocyte cell lines. *Proc Natl Acad Sci USA* **86**:7200-7203, 1989.

Note: Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: U38 from Dr. Barbara K. Felber and Dr. George N. Pavlakis." Also include the references cited above in any publications.

An NCI patent application has been filed on the use of the cell line U38. Corporate requests should be directed in writing to: B.K. Felber or G.N. Pavlakis, National Cancer Institute, FCRDC, ABL-Basic Research Program, P.O. Box B/Building 539, Room 121, Frederick, Maryland 21702-1201. Phone: (301) 846-1474, FAX (301) 846-5991.

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.



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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:

osteosarcoma 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 (Roche 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]

Related Links



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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. <i>Nature</i> 319: 511-514, 1986. PubMed: 3456080 22509: Ponten J, Saksela E. Two established in vitro cell lines from human mesenchymal tumours. <i>Int. J. Cancer</i> 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. <i>J. Cell. Physiol.</i> 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. <i>Cancer Res.</i> 57: 3562-3568, 1997. PubMed: 9270029 32308: Moradpour D, et al. Characterization of cell lines allowing tightly regulated expression of hepatitis C virus core protein. <i>Virology</i> 222: 51-63, 1996. PubMed: 8806487

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

Product code 500320
Product name EM STBL4

Company/Undertaking Identification

INVITROGEN CORPORATON
 5791 VAN ALLEN WAY
 PO BOX 6482
 CARLSBAD, CA 92008
 760-603-7200



INVITROGEN CORPORATION
 5250 MAINWAY DRIVE
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 301-431-8585
 Outside of the U.S. ++1-301-431-8585

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2. COMPOSITION/INFORMATION ON INGREDIENTS
--

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	7-13

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview

The product contains no substances which at their given concentration, are considered to be hazardous to health
May be harmful if swallowed
May cause skin and eye irritation in susceptible persons

Form
Liquid

Principle Routes of Exposure/

Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	No information available
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

No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
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 No special handling advice required
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)
Glycerol	15 mg/m ³ total dust 5 mg/m ³ respirable fraction	-	10 mg/m ³	-

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 No data available °F No data available

Melting point/range °C No data available °F No data available

Flash point °C No data available °F No data available

Autoignition temperature °C No data available °F No data available

Oxidizing properties No information available

Water solubility No data available

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)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m ³ (Rat)

Principle Routes of Exposure/

Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	No information available
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

(Long Term Effects)

Target Organ Effects

No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	No information available.
Bioaccumulation	No information available

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

15. REGULATORY INFORMATION

International Inventories

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

U.S. Federal Regulations

SARA 313

This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

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

For research use only

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 present unknown hazards and should be used with caution. Since the Company 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, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet



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Cell Biology

ATCC® Number: CRL-1715™ [Order this Item](#) Price: \$329.00

Designations: TM4

Depositors: JP Mather

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: epithelial

Source: **Organ:** testis
Disease: normal
Cell Type: Sertoli cell;

Cellular Products: retinol binding protein
tissue plasminogen activator
transferrin

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 Transfection Reagents)

Receptors: follicle stimulating hormone (FSH), expressed
androgen receptor, expressed
progesterone receptor, expressed

Tumorigenic: No

Antigen Expression: H-Y antigen; *Mus musculus*, expressed

Age: 11 to 13 days

Gender: male

Comments: The TM4 cell line is reported to respond to FSH with an increase in cAMP production, but to not respond to luteinizing hormone (LH). The FSH responsiveness is much reduced compared to primary sertoli cell cultures. Constitutive plasminogen activator production is reported to be low, but is stimulated by FSH and, to a greater extent, by retinoic acid.
Tested and found negative for ectromelia virus (mousepox).

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Propagation:

ATCC complete growth medium: A 1:1 mixture of Ham'S F12 medium and Dulbecco's modified Eagle's medium with 1.2 g/L sodium bicarbonate and 15 mM HEPES, 92.5%; horse serum, 5%; fetal bovine serum, 2.5%

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 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.

Subcultivation Ratio: A subcultivation ratio of 1:10 to 1:50 is recommended

Medium Renewal: Every 3 to 4 days

Preservation:

Freeze medium: Culture medium, 95%; DMSO, 5%

Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2006](#)
recommended serum: [ATCC 30-2020](#)
recommended serum: [ATCC 30-2040](#)

References:

1158: Mather JP. Establishment and characterization of two distinct mouse testicular epithelial cell lines. *Biol. Reprod.* 23: 243-252, 1980. PubMed: [6774781](#)

1159: Mather JP, et al. Culture of testicular cells in hormone-supplemented serum-free medium. *Ann. N.Y. Acad. Sci.* 383: 44-68, 1982. PubMed: [7046561](#)

1184: Carson DD, et al. Synthesis and secretion of a novel binding protein for retinol by a cell line derived from Sertoli cells. *J. Biol. Chem.* 259: 3117-3123, 1984. PubMed: [6538197](#)

26150: Mather JP, Phillips DM. Establishment of a peritubular myoid-like cell line and interactions between established testicular cell lines in culture. *J. Ultrastruct. Res.* 87: 263-274, 1984. PubMed: [6544874](#)

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Technical Data Sheet

Reagent: Cynomolgus T cell line

Clone: HSC-F

Lot: Dec 2007

Species: *Macaca fascicularis*

Description: Immortalized cell line derived by transformation of cynomolgus monkey fetal splenocytes with *Herpesvirus saimiri* (1). IL-2-independent. Cell line kindly provided by Dr. Hirofumi Akari, Tsukuba Primate Research Center and the Health Science Research Resources Bank, Osaka, Japan

Supplied as: 10^7 cells in 90% FBS, 10% DMSO, shipped on dry ice

Medium: 90% RPMI 1640 with L-glutamine, 10% FBS and penn/strep

Doubling time: 30 hours

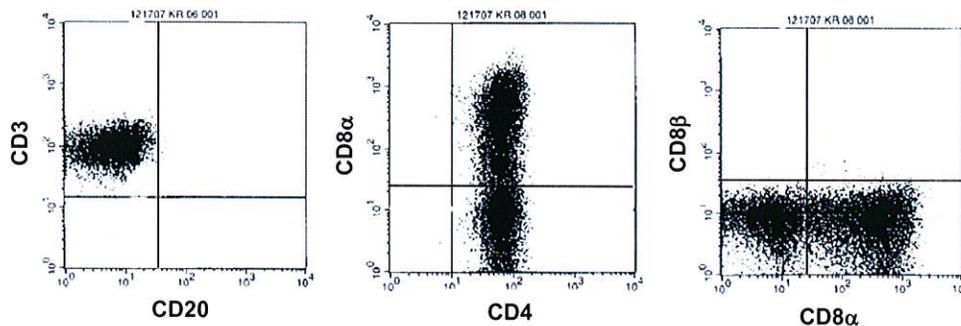
Instructions for propagation: Cells grow as small clumps largely in suspension culture. Split cultures 1-2 times weekly as needed.

BIOHAZARD: It is unknown whether this cell line is productively infected with *Herpesvirus saimiri*. *H. saimiri* has been classified as NIH Risk Group I*.



*Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. U.S Government Printing Office, Washington: 2007.

Immunophenotype: CD3+ (100%), CD4+ (100%), CD8 α + (~50%), CD8 β - (0%), CD20- (0%)



References: (1) Akari H, Nam KH, Mori K, Otani I, Shibata H, Adachi A, Terao K, Yoshikawa Y. Effects of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. *Clin Immunol.* 1999; 91(3):321-9.

For Research Use Only- All reagents are to be used in accordance with the terms of the Resource registration agreement.



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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)

Morphology: lymphoblast



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 (Roche 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 neurotoxin 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 in CD4+ T lymphocytes. *Proc. Natl. Acad. Sci. USA* 93: 7695-7700, 1996. PubMed: [8755538](#)
- 32922: Yang RY, et al. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc. Natl. Acad. Sci. USA* 93: 6737-6742, 1996. PubMed: [8692888](#)
- 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](#)
- 33025: Ponton A, et al. The CD95 (APO-1/Fas) receptor activates NF-kappaB independently of its cytotoxic function. *J. Biol. Chem.* 271: 8991-8995, 1996. PubMed: [8621545](#)
- 33042: August A, Dupont B. Association between mitogen-activated protein kinase and the zeta chain of the T cell receptor (TcR) with the SH2,3 domain of p56lck. *J. Biol. Chem.* 271: 10054-10059, 1996. PubMed: [8626561](#)
- 33122: Kotanides H, Reich NC. Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. *J. Biol. Chem.* 271: 25555-25561, 1996. PubMed: [8810328](#)
- 33136: Hartley D, Corvera S. Formation of c-Cb1-phosphatidylinositol 3-kinase complexes on lymphocyte membranes by a p56lck-independent mechanism. *J. Biol. Chem.* 271: 21939-21943, 1996. PubMed: [8702998](#)
- 33167: Chen H, et al. Octamer binding factors and their coactivator can activate the murine PU.1 (spi-1) promoter. *J. Biol. Chem.* 271: 15743-15752, 1996. PubMed: [8663022](#)
- 50685: Schneider U, et al. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *Int. J. Cancer* 19: 621-626, 1977. PubMed: [68013](#)
- 112530: Ronald Wange, personal communication

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[Print this Page](#)**Cell Biology**

ATCC® Number:	TIB-161™	Order this Item	Price:	\$279.00
Designations:				
Depositors:	AF Gazdar			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	suspension			
Organism:	<i>Homo sapiens</i> (human)			
Morphology:	lymphoblast			



Source:	Disease: Sezary Syndrome Cell Type: cutaneous T lymphocyte;
Cellular Products:	interleukin 2 [1140] tumor necrosis factor alpha (TNF alpha) [23420]
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
Receptors:	interleukin 2 (IL-2), expressed [1140]
Tumorigenic:	Yes
Antigen Expression:	CD4; Homo sapiens [22610]
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11,12 D5S818: 11,12 D7S820: 8,11 THO1: 8,9 TPOX: 8,9 vWA: 14,15
Age:	53 years adult
Gender:	male
Ethnicity:	Caucasian

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Comments: H9 (ATCC HTB-176) is a clonal derivative of HuT 78 [PubMed: 2567177].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 20%.
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Cultures can be maintained by addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension in fresh medium at 2 X 10 exp5 viable cells/ml. Maintain cultures at cell concentrations between 5 X 10 exp4 and 8 X 10 exp5 viable cells/ml. maintain cell density at less than 1 X 10 exp6 cells/ml.
Medium Renewal: Two to three times weekly

Preservation: **Freeze medium:** Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Doubling Time: about 65 hours

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2005
recommended serum:ATCC 30-2020
derivative:ATCC HTB-176

References: 1140: Gootenberg JE, et al. Human cutaneous T cell lymphoma and leukemia cell lines produce and respond to T cell growth factor. J. Exp. Med. 154: 1403-1418, 1981. PubMed: 6975346
22484: Mann DL, et al. Origin of the HIV-susceptible human CD4+ cell line H9. AIDS Res. Hum. Retroviruses 5: 253-255, 1989. PubMed: 2567177
22610: Gazdar AF, et al. Mitogen requirements for the in vitro propagation of cutaneous T-cell lymphomas. Blood 55: 409-417, 1980. PubMed: 6244013
23228: Chen TR. Karyotypic derivation of H9 cell line expressing human immunodeficiency virus susceptibility. J. Natl. Cancer Inst. 84: 1922-1926, 1992. PubMed: 1460674
23420: O'Connell MA, et al. Cellular proliferation and activation of NF kappa B are induced by autocrine production of tumor necrosis factor alpha in the human T lymphoma line HuT 78. J. Biol. Chem. 270: 7399-7404, 1995. PubMed: 7706285
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
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
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

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Cell Biology

ATCC® Number: **CRL-7131™** [Order this Item](#)

Price: **\$429.00**

Designations: **Hs 181.Tes**
 Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Homo sapiens* (human)
 Morphology: fibroblast

Source: **Organ:** testis
Disease: normal

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.

DNA Profile (STR): Amelogenin: X,Y
 CSF1PO: 13
 D13S317: 8,13
 D16S539: 12
 D5S818: 8,12
 D7S820: 10,11
 THO1: 6,9
 TPOX: 8,11
 vWA: 19

Cytogenetic Analysis: modal number = 46; range = 45 to 47

Age: 14 weeks gestation

Gender: male

Ethnicity: Caucasian

Comments: Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC . We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description.

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:** **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: Every 2 to 3 days
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 medium (without the additional supplements or serum described under ATCC Medium):[ATCC 30-2002](#)
recommended serum:[ATCC 30-2020](#)

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Cell Biology

ATCC® Number: CRL-1543™ [Order this Item](#) **Price:** \$329.00

Designations: HOS

Depositors: JS Rhim

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: mixed, fibroblast and epithelial like cells

Source: **Organ:** bone
Disease: osteosarcoma

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.

DNA Profile (STR): Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 10,13
D5S818: 13
D7S820: 11,12
THO1: 6
TPOX: 8,11
vWA: 18

Isoenzymes: G6PD, B

Age: 13 years

Gender: female

Ethnicity: Caucasian

Comments: HOS cells exhibit flat morphology, low saturation density, low plating efficiency in soft agar and are sensitive to chemical and viral transformation.

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

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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 1:2 to 1:4 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](#)

References:

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Cell Biology

ATCC® Number: CCL-2™ [Order this Item](#)

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] Roche 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

Price: \$279.00

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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 M1, 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 (CO ₂), 5% Temperature: 37.0°C
Subculturing:	Protocol: <ol style="list-style-type: none">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 also available as Certified Reference Material, ATCC CRM-CCL-2 recommended serum: ATCC 30-2020 derivative: ATCC CCL-2.1 derivative: ATCC CCL-2.2 derivative: ATCC CCL-2.3

References:

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Cell Biology

ATCC® Number:	CRL-2996™	Order this Item	Price:	\$379.00
Designations:	EML-3C			Related Links ▶ NCBI Entrez Search Cell micrograph Make a Deposit Frequently Asked Questions Material Transfer Agreement Technical Support Related Cell Culture Products Login Required ▶ Product Information Sheet
Depositors:	R Montelaro			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	Equus caballus (horse)			
Morphology:	macrophage-like			



Source:	Organ: peripheral blood Cell Type: macrophage-like Breed: Portuguese autochthonous (Garrano)
Cellular Products:	non-specific esterase produces nitrites in response to LPS stimulation
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: May 2005 limiting dilution
Applications:	study of interaction between EIAV and macrophages; lentiviruses persistence; cytopathicity mechanisms
Receptors:	equine infectious anemia virus (EIAV) cellular receptor, expressed
Virus Susceptibility:	Equine infectious anemia virus
Antigen Expression:	CZ2.2+, Ki-M6 (CD68)+, MHC-I+, MHC-II+
Age:	2 years old
Gender:	male

Comments: The EML-3C cell line was established in 2005 from isolated peripheral blood mononuclear cells of a two-year-old male Portuguese Garrano horse. These cells possess functional properties of macrophages such as non-specific esterase (NSE) activity, are able to phagocytose fluorescent bioparticles and produce nitrites in response to lipopolysaccharide (LPS) stimulation. The EML-3C cell line expresses the EIA V receptor for cellular entry (ELR1) and supports replication of the virulent equine infectious anemia virus (EIA V_{pv}). This cell line can be used as a valuable tool for studying equine macrophage functions, lentivirus infection, and the equine immune system. [16173840]

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 500 ml of the base medium:

- fetal bovine serum (FBS) to a final concentration of 10%
 - horse serum (HS) to a final concentration of 10%
 - 0.1 mM non-essential amino acids

Temperature: 37.0°C

Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

Subculturing: **Protocol:** Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS) or 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 37.0°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10⁴ to 4 X 10⁴ viable cells/cm² is recommended.
7. Incubate cultures at 37.0°C.
Subcultivation ratio: A subcultivation ratio of 1:4 to 1:8 is recommended.
Medium renewal: Every 2 to 3 days

Preservation: **Freeze medium:** complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2002
Recommended fetal bovine serum: ATCC 30-2020
Recommended horse serum: ATCC 30-2040
Trypsin EDTA Solution: ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X
MEM Non-Essential Amino Acid Solution, 100x, ATCC 30-2116

References: 16173840: Isabel Fidalgo-Carvalho et al. Characterization of an equine macrophage cell line: application to studies of EIAV infection. Veterinary Microbiol. 136 (1-2): 87-99, 2009. PubMed: 19038510

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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 ([Roche 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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Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Cercopithecus aethiops</i>			
Morphology:	fibroblast			
Source:	Organ: kidney Cell Type: SV40 transformed			

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Cellular Products: T antigen

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Applications: transfection host (Roche 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. The cells contain a single integrated copy of the complete early region of the SV40 genome.

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

Subculturing:**Protocol:**

1. Remove and discard 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 5 to 10 min).
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.

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 95%; DMSO, 5%

Storage temperature: liquid nitrogen vapor temperature

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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NIH AIDS Research & Reference Reagent Program

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Building 6, Suite 200
Germantown, MD 20874
USA

Phone: 240-686-4740
Fax: 301-515-4015
aidsreagent.org

DATA SHEET

Reagent: CEM-SS

Catalog Number: 776

Lot Number: 15 070569

Release Category: C

Provided: 1.3×10^7 cells/mL. Viability is 96%.

Propagation Medium: RPMI 1640, 89%; PSN antibiotics (Gibco), 1%; fetal bovine serum, 10%.

Freeze Medium: RPMI 1640, 66%; fetal bovine serum, 27%; DMSO, 7%.

Growth Characteristics: These cells double approximately every 1-2 days and grow as a suspension of single or small (3-10 cell) aggregates. The cells are optimally maintained on a rocker platform or roller bottle apparatus and can be split at 1:20 one to two times per week.

Morphology: Generally a round, individual, slightly refractile cell population that occasionally forms small aggregates as observed under normal culture conditions. Small numbers of individual highly refractile karyocytomegalic cells may also be observed.

Special Characteristics: These cells have been cloned for both poly-L-lysine induced adherence to microtiter plates and viral-induced syncytial/fusogenic sensitivity following infection with either cell-free or cell-associated HIV-1 and HIV-2. Cells are negative for any virus including human retroviruses as determined by electron microscopy and reverse transcriptase analysis. They can be used for virus production, aspects of HIV-1 cell fusion and molecular biology studies and for the analysis of infectivity, antiviral agents and neutralizing antibodies in the assays referenced below.

[CEM-SS Microtiter Syncytial-Forming Assay](#)

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Sterility: Negative for bacteria, mycoplasma, and fungi.

Recommended Storage: Liquid nitrogen.

Contributor: Dr. Peter L. Nara.

Description: Human T4-lymphoblastoid cell line initially derived by G.E Foley et al. and biologically cloned by P.L. Nara et al.

References:
Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18**:522-529, 1965.
Nara PL, Hatch WC, Dunlop NM, Robey WG, Fischinger PJ. Simple, rapid quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *AIDS Res Hum Retroviruses* **3**:283-302, 1987.
Nara PL, Fischinger PJ. Quantitative infectivity assay for HIV-1 and -2. *Nature* **332**:469-470, 1988.

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM-SS (Cat# 776) from Dr. Peter L. Nara." Please include the references cited above in any publications.

Last Updated August 12, 2010

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[Print this Page](#)**Cell Biology**

ATCC® Number: CRL-11268™ [Order this Item](#) **Price:** \$279.00

Designations: 293T/17 [HEK 293T/17]

Depositors: Rockefeller Univ.

Biosafety Level: 2 [Cells contain Adeno and SV-40 viral DNA sequences]

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source: **Organ:** kidney

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Restrictions: The line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.

Antigen Expression: SV40 T antigen [45408]

DNA Profile (STR): Amelogenin: X
CSF1PO: 11, 12
D13S317: 12, 14
D16S539: 9, 13
D5S818: 8, 9
D7S820: 11
THO1: 7, 9.3
TPOX: 11
vWA: 16, 18, 19

Age: fetus

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Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability. 293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line. ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line. ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.
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 (CO ₂), 5%
Subculturing:	Protocol: <ol style="list-style-type: none"> 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. <p>Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended Medium Renewal: Every 2 to 3 days</p>
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-11269
References:	45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361 57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999 57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001 57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

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Plasmid Maps



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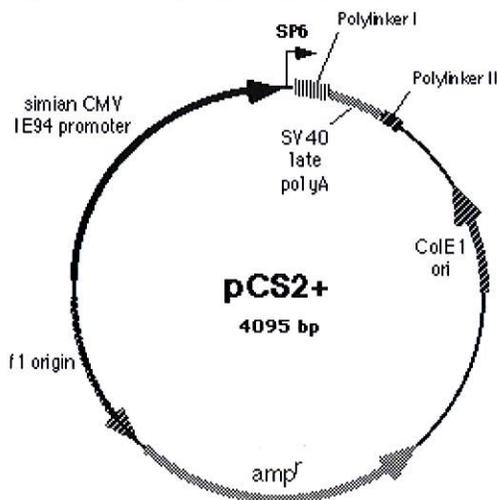
URL to bookmark for this site: <http://sitemaker.umich.edu/dlturner.vectors>

Sequences and maps for SIBR vectors are now available (see link to left)

CS2+ vector

pCS2+ is a multipurpose expression vector. Although originally designed for expressing proteins in *Xenopus* embryos from either injected RNA or DNA, pCS2+ is also useful for high-level transient expression in a wide variety of mammalian and avian cells. It is also functional in zebrafish embryos (as DNA or RNA), and it can be used for in vitro transcription/translation (using, for example, the Promega TnT system). A number of derivatives of CS2 have been constructed that allow fusions to epitope tags and other marker proteins, as well as nuclear localization signals or the gal4 DNA binding and activation domains. In almost all cases, the same reading frames are used for the fusion vectors, to facilitate moving genes between multiple CS2 derivatives.

pCS2 features: pCS2+ contains a strong enhancer/promoter (simian CMV IE94) followed by a polylinker and the SV40 late polyadenylation site. An SP6 promoter is present in the 5' untranslated region of the mRNA from the sCMV promoter, allowing in vitro RNA synthesis of sequences cloned into the polylinker. A T7 promoter in reverse orientation between the polylinker and the SV40 polyA site for probe synthesis, as well as a second polylinker after the SV40 polyA site to provide several possible sites to linearize the vector for SP6 RNA transcription. The vector backbone is from pBluescript II KS+ and includes the amp resistance gene and an f1 origin for producing single stranded DNA.



Contact Information:

Dave Turner
E-mail: dlturner@umich.edu
Phone: 734-647-6890
Fax: 734-936-2690

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Location of features

- Tet-responsive promoter $P_{hCMV^{*1}}$: 7–439
Tet response element (TRE)
Location of seven *tetO* 19-mers: 7–319
Fragment containing $P_{minCMV^{*}}$: 320–439
TATAA box: 342–349
- HA tag: 505–537
- Multiple cloning site (MCS): 546–600
- Fragment containing β -globin poly-A signal: 608–1774
- Fragment containing Col E1 origin of replication: 1975–2619
- Ampicillin resistance gene (β -lactamase):
Start codon (ATG): 3429–3427; stop codon: 2769–2767
- Hygromycin resistance gene: 3838–5392
 P_{SV40} promoter: 3838–4108
Hygromycin coding sequence: 4175–5200
SV40 poly-A signal: 5338–5392

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers ampicillin resistance (100 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. New Tet Vectors: pTRE2pur & pTRE2hyg (October 2000) *Clontechiques* **XV**(4):20.
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.

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 Laboratories, Inc. This vector has not been completely sequenced.

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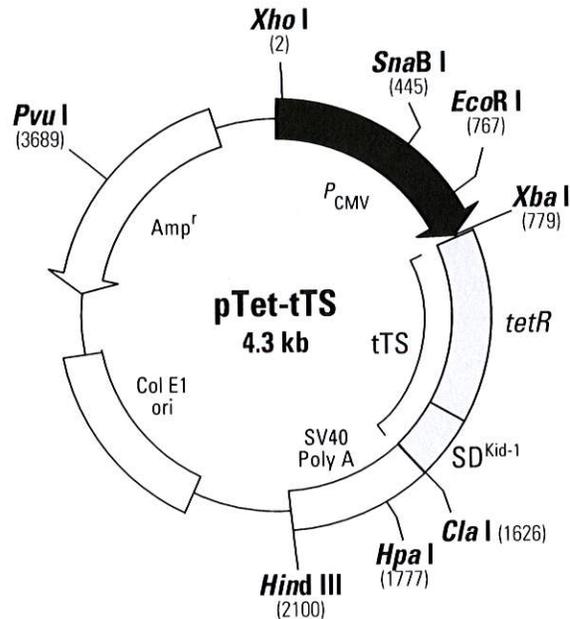
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pTet-tTS Vector Information

GenBank Accession No.: Submission in progress.

PT3334-5

Cat. No. 631011



Restriction map of pTet-tTS Vector. All restriction sites shown are unique.

Description:

The pTet-tTS Vector, designed for use with the Tet-On™ Gene Expression System, prevents unregulated gene expression in the absence of the inducing agent Doxycycline (Dox).

pTet-tTS helps overcome one of the major limitations of regulated gene expression in mammalian systems: low-level background expression. In transient transfections, background expression can result from the high copy number of the introduced plasmid and lack of chromatin repression. In stable cell lines, the level of background expression is dependent on where the gene of interest integrates into the genome. If the expression construct integrates too close to an enhancer element, for instance, unregulated expression may occur.

pTet-tTS encodes the tetracycline-controlled transcriptional silencer (tTS), which is a fusion of the tet repressor protein (TetR) and the KRAB-AB silencing domain of the Kid-1 protein (SD^{Kid-1}), a powerful transcriptional repressor (1, 2). In the *absence* of Dox, tTS binds to the *tetO* sequence in the tet-response element (TRE) region of the Tet response plasmid (pTRE2 or pRevTRE) and blocks expression of the gene of interest. As Dox is added to the culture medium, the tTS dissociates from the TRE, relieving transcriptional suppression. At sufficient Dox concentrations, the rtTA transactivator encoded by the pTet-On™ Vector binds to the TRE, thus activating expression of the gene of interest. By silencing unregulated transcription in the *absence* of Dox, the tTS provides complete on/off control of gene expression in either stable or transient systems—regardless of integration site or copy number of the response plasmid.

Use:

tTS will bind to the TRE in the presence of 0–10 ng/ml of Dox. It begins to dissociate from the TRE at concentrations >10 ng/ml. rtTA will bind to the TRE at >100 ng/ml of Dox. We recommend a working Dox concentration of 1 µg/ml for inducing gene expression.

pTet-tTS can be introduced in one of three ways: during the establishment of a Tet-On cell line; after establishing a Tet-On cell line and before introducing the response plasmid; or during introduction of the response plasmid. The last option is recommended if you are introducing a potentially toxic gene. pTet-tTS should only be used with the Tet-On Gene Expression System. It is not compatible for use with the Tet-Off™ System.

For introduction during establishment of Tet-On cell lines

pTet-tTS does not contain a drug-resistance marker for clonal selection in mammalian cell culture. Therefore, we recommend cotransfecting pTet-tTS with the pTet-On Vector, which contains the rtTA and the neomycin-resistance gene. Transfections should be performed at a 1:10 molar ratio (i.e., 1 μ g pTet-On to 10 μ g pTet-tTS).

If you have already established a Tet-On cell line

First, cotransfect pTet-tTS with a plasmid containing a selectable marker (such as pTK-Hyg, Cat. No. 631750) at a 1:10 molar ratio (i.e., 1 μ g selection marker to 10 μ g pTet-tTS). After selecting stable cell lines, transfect the pTRE response plasmid.

If you are infecting cells with a potentially toxic gene

Create a Tet-On cell line first, then cotransfect these vectors in the following ratios:

Selection marker : (pTK-Hyg)	pTRE-toxic gene :	pTet-tTS
1 (50–100 ng)	10 (0.5–1.0 μ g)	50–100 (5–10 μ g)

Location of features:

- Fragment containing P_{CMV} promoter: 86–673
- tTS transcriptional silencer:
 - Start codon: 775–777; stop codon: 1621–1623
 - Tet Repressor (TetR): 775–1407
 - SV40 nuclear localization sequence: 1408–1425
 - KRAB-AB Silencing Domain (SD^{Kid-1}): 1426–1623
- Fragment containing SV40 poly-A sequence: 1647–2104
- Col E1 origin of replication: 2454–3097
- Ampicillin resistance (β -lactamase) gene:
 - Start codon: 4105–4103; stop codon: 3247–3245

Propagation in *E. coli*:

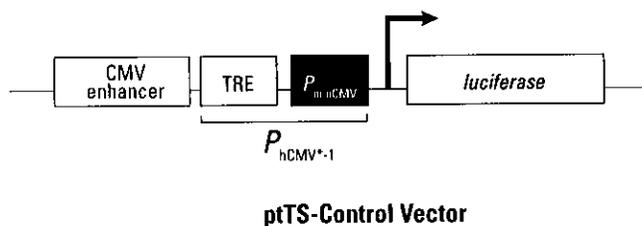
- Suitable host strains: DH5 α , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

ptTS-Control Vector:

The ptTS-Control Vector is provided to simplify functional testing of clones expressing tTS. ptTS-Control contains the firefly luciferase gene as the reporter, which is controlled by the tet-response element (TRE). Unlike pTRE2 or other TRE-based vectors available from Clontech, ptTS-Control contains the CMV enhancer located upstream of P_{hCMV-1} to drive high level background expression. Essentially, this vector construct mimics chromosomal integration of pTRE Vector near an endogenous enhancer sequence. In the absence of doxycycline, functional tTS binds the TRE, thus blocking enhancer activity. This action results in low basal activity and high inducibility of reporter gene expression.

Use of control vector:

To test the function of tTS in stable cell lines, transfect ptTS-Control Vector into the cell line by any standard method. Prepare parallel transfections to test tTS in the presence and absence of Dox. In the absence of Dox, tTS will bind to the TRE of ptTS-Control Vector, resulting in low-level gene expression. Conversely, in the presence of Dox (i.e., 1 μ g/ml), tTS will dissociate from TRE, allowing the CMV enhancer to drive expression of the reporter gene. Luciferase is a highly sensitive enzymatic reporter that can be assayed by any standard luciferase-detection method.



Schematic map of ptTS-Control Vector.

References:

1. Freundlieb, S., *et al.* (1999) *J. Gene Med.* 1:4–12.
2. Witzgall, R., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:4514–4518.

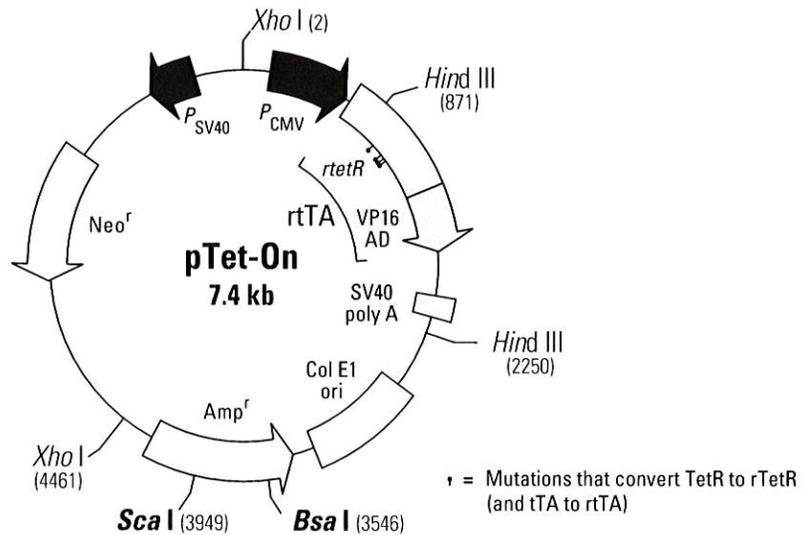
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Restriction Map of pTet-On® Vector. Unique restriction sites are in bold

Description

pTet-On® expresses the reverse tet-responsive transcriptional activator (rtTA) 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-On is similar to pTet-Off except for four amino acid changes that convert the TetR to a rTetR, and consequently, tTA to rtTA. (pTet-Off also contains several silent mutations.) pTet-On was originally described as pUHD17-1neo by Gossen *et al.* (1995). pTet-On can be distinguished from pTet-Off by digestion with *Hind* III.

Use

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

Location of Features

- Fragment containing P_{CMV} : 86–673
- reverse tetracycline-responsive transcriptional activator (rtTA): 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.

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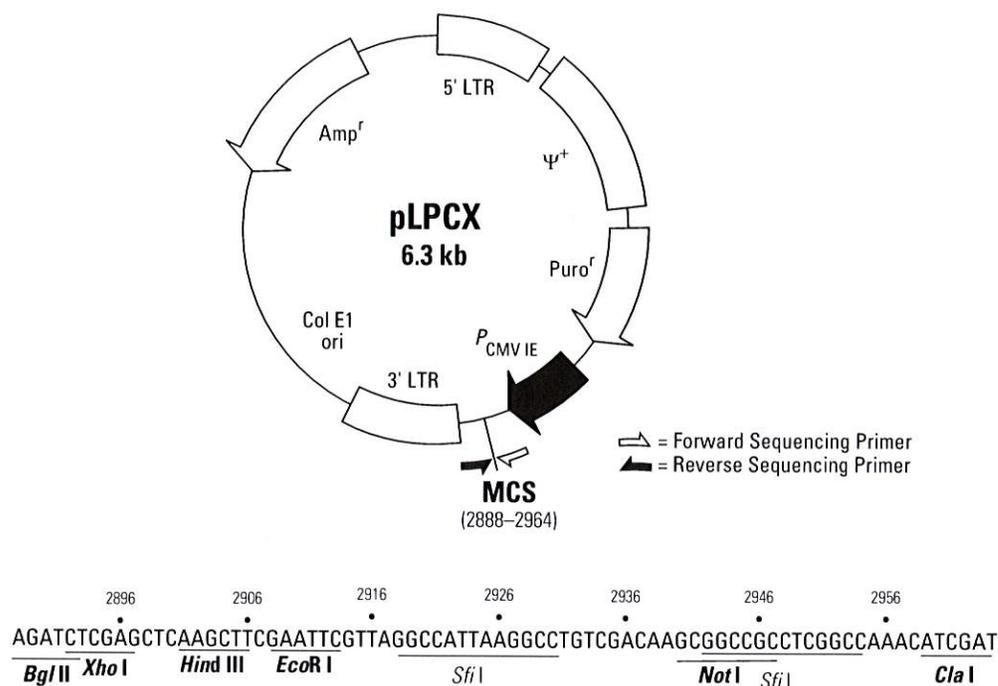
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pLPCX Vector Information

PT3299-5

GenBank Accession #: Submission in progress.

Sold as part of Catalog #K1061-1



Restriction Map and Multiple Cloning Site (MCS) of pLPCX. Unique restriction sites are shown in bold.

Description

pLPCX contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression (1–3). Upon transfection into a packaging cell line, pLPCX transiently expresses, or integrates and stably expresses, a transcript containing Ψ^+ (the extended viral packaging signal) the puromycin resistance gene, and the gene of interest. The 5' viral LTR in this vector contains promoter/enhancer sequences that control expression of the puromycin resistance ($Puro^r$) gene for antibiotic selection in eukaryotic cells. A gene of interest can be cloned into the multiple cloning site immediately downstream of the human cytomegalovirus (CMV) immediate early promoter ($P_{CMV IE}$). pLPCX also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pLPCX can be transfected into a packaging cell line such as the RetroPack™ PT67 Cell Line (#K1060-D). Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLPCX does not contain the structural genes (*gag*, *pol*, and *env*) necessary for particle formation and replication; however, these genes are stably integrated into PT67 (4–7). Subsequent introduction of pLPCX, containing Ψ^+ , transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. These retroviral particles can infect target cells and transmit the gene of interest (which is cloned between the viral LTR sequences), but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^* (extended packaging signal): 659–1468
Mutated *gag* (ATG→TAG): 1049–1051
- Puromycin resistance gene (Puro^r):
Start codon: 1566–1568; stop codon: 2163–2165
- Immediate early CMV promoter ($P_{CMV IE}$): 2338–2868
- Multiple Cloning Site (MCS): 2888–2964
- 3' MoMuLV LTR: 3004–3597
- Col E1 origin of replication:
Site of replication initiation: 4266
- Ampicillin resistance gene (β -lactamase):
Start codon: 5886–5884; stop codon: 5028–5026

Sequencing Primer Locations

- pLNCX Seq/PCR Primers (#K1060-F)
5' primer (2844–2868): 5'-AGCTCGTTTAGTGAACCGTCAGATC-3'
3' primer (3026–3001): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*

- Suitable host strains: DH5 α , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* 7:980–990.
4. Mann, R., *et al.* (1983) *Cell* 33:153–159.
5. Miller, A. D. & Baltimore, C. (1986) *Mol. Cell. Biol.* 6:2895–2902.
6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* 18:3587–3590.
7. Miller, A. D. & Chen, F. (1996) *J. Virol.* 70:5564–5571.

Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

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.

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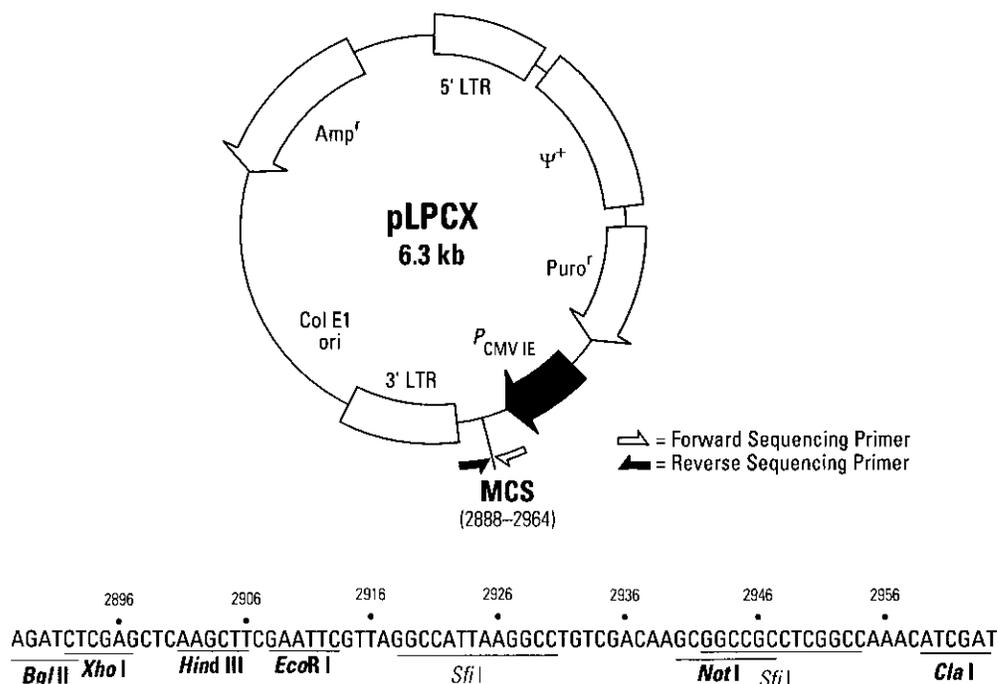
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pLPCX Vector Information

PT3299-5

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Sold as part of Catalog #K1061-1



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- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
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Product: Expression Arrest™ pLKO.1 empty vector

Catalog #: RHS4078

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Huntsville, AL 35806 /F/ 256.704.4849

The Expression Arrest-TRC shRNA libraries are the result of a collaborative research effort based at the Broad Institute of MIT and Harvard, and include six MIT and Harvard associated research institutions and five international life sciences organizations. The goal of the TRC is to create lentiviral shRNA libraries targeting 15,000 human and 15,000 mouse annotated genes with multiple constructs per gene. Open Biosystems is a distribution partner with the TRC to make these shRNA libraries available to researchers worldwide.

Features of the TRC shRNA libraries include:

- siRNA rules-based design for efficient gene knockdown
- Already cloned into lentiviral vectors
- Amenable to *in vitro* and *in vivo* applications such as the creation of stable knockdowns
- Lentiviral vector enables transduction of primary and non-dividing cell lines
- Broad coverage: 4-5 constructs per gene

The TRC shRNA constructs are designed to include a hairpin of 21 base-pair sense and antisense stem and a 6 base-pair loop. Each hairpin sequence was cloned into the lentiviral vector (pLKO.1) and sequence verified. Multiple constructs (4-5) were created per gene to ensure adequate coverage of the target gene.

The pLKO.1 Control Vector (See Figure 1) is a negative control for any transfection experiment performed using the Expression Arrest-TRC shRNA-containing expression vectors. This control is the pLKO.1 empty vector without a hairpin insert.

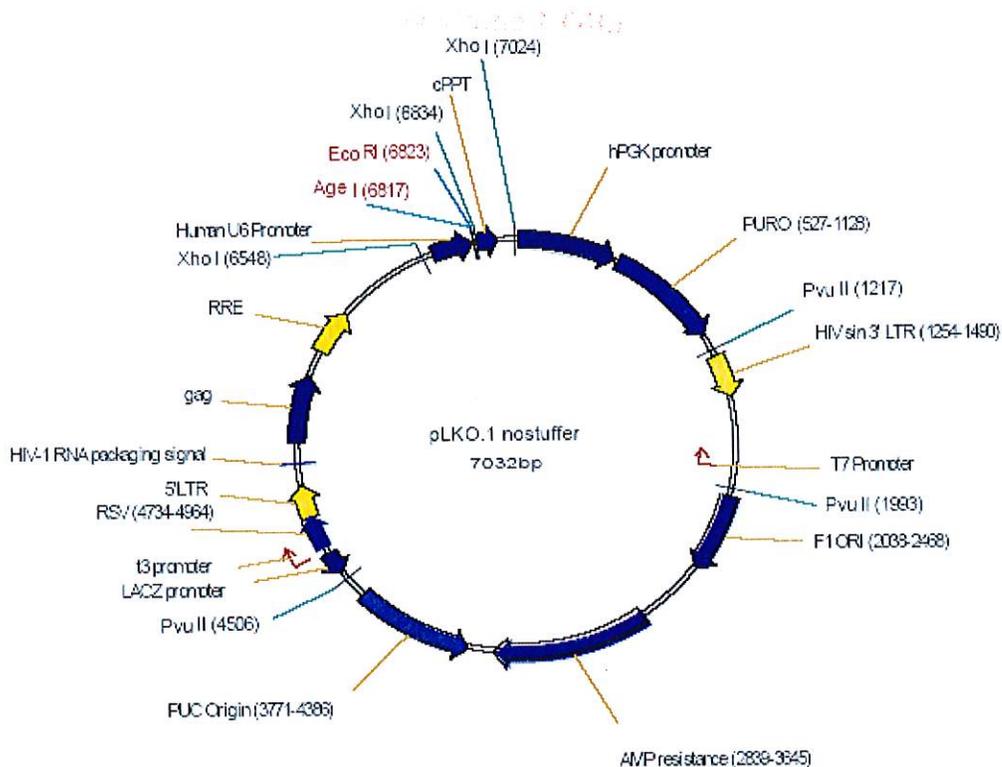
Shipping

Each vial of the pLKO.1 control vector is shipped at a concentration of 0.5µg/µl in a total volume of 20µl, thus providing a total amount of 10µg vector DNA.

pLKO.1 vector storage

The vector DNA is shipped in a microfuge tube at room temperature and should be stored at -20°C or -80°C.

Figure 1: Vector Map of pLKO.1



VECTOR ELEMENT	UTILITY
Human U6 Promoter	RNA generated with four uridine overhangs at each 3' end
hPGK	Human phosphoglycerate kinase promoter
PuroR	Puromycin mammalian selectable marker
3' SIN LTR	3' Self inactivating long terminal repeat
f1 ori	f1 origin of replication
AmpR	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
RRE	Rev response element
cPPT	Central polypurine tract

Antibiotic Resistance

Table 1: Antibiotic Resistance Conveyed by pLKO.1

Antibiotic	Concentration	Utility
Ampicillin (Carbenicillin)	100µg/ml	Bacterial selection marker
Puromycin		Mammalian selectable marker

The pLKO.1 expression vector is a self-inactivating (SIN) lentiviral vector, containing a deletion in the U3 region of the 3' LTR. Self-inactivating lentiviral particles can be produced by transfection into lentiviral packaging cell lines.

The pLKO.1 plasmid DNA provided is of high purity and is validated transfection ready therefore it can be used immediately in RNAi assays.

Restriction Digests of pLKO.1

You may wish to restriction digest a sample of your plasmid DNA following plasmid DNA preparation. The following is a protocol for dual restriction enzyme digestion using BamH1 and Nde1 for quality control of pLKO.1 vectors.

Using filtered pipette tips and sterile conditions add the following components, in the order stated, to a sterile PCR thin-wall tube.

Sterile, nuclease-free water	14.8µl
Restriction enzyme BamH1	1µl
Restriction enzyme BamH110X buffer	2µl
BSA (10X, 10mg/ml)	0.2µl
DNA sample 1µg, in water or TE buffer	1µl
Restriction enzyme Nde1 20U	1µl
Final volume	20µl

2. Mix gently by pipetting.
3. Incubate in a thermalcycler at 37°C for 2.5 hours to digest then at 70°C for 20 minutes to denature the enzyme.
4. Add 4µl of 6X loading dye (or another appropriate DNA loading buffer), and proceed to gel analysis.
5. Load the gel with 20µl of the digested samples on a 1% agarose gel. Also run 1µl (1µg) of the uncut sample combined with 16µl of water and 3µl of 6x dye alongside the digested samples.
6. The digest will produce two fragments one approximately 7-kb band and a 749bp band

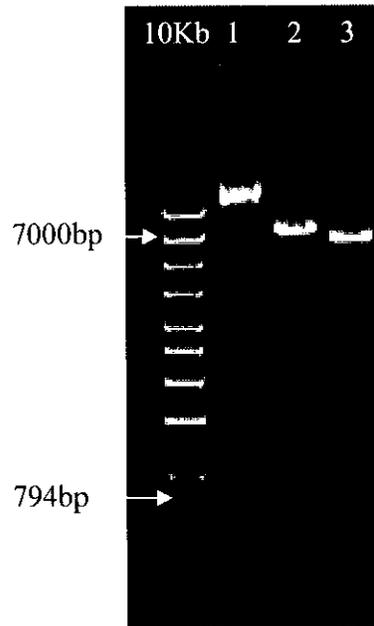
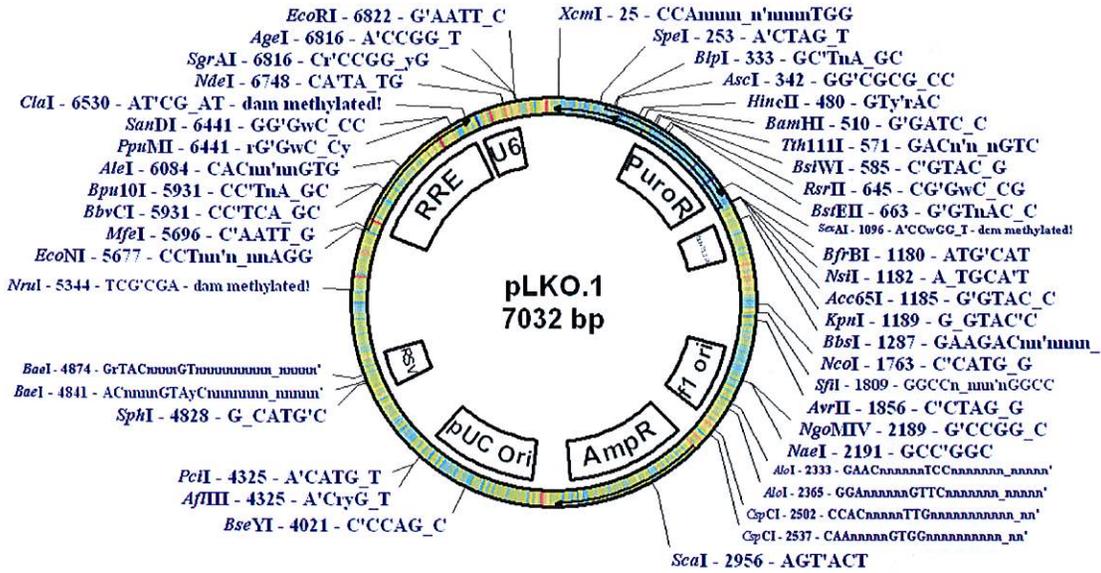


Figure 2. The 1% agarose gel above contains -10kb ladder followed by undigested sample and restriction digests of pLKO.1 vector (labeled 1-3), The lanes are loaded as follows: 1 - Uncut plasmid 2 - Cut with BamHI. Expected to linearize at 7032bp. 3 - Cut with BamHI and NdeI. Band sizes of 6238bp and 794bp expected.

Figure 3: Single cutters for pLKO.1



Vector sequence

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Useful References:

Stewart, S.A., *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA*, **9**, 493-501 (2003).

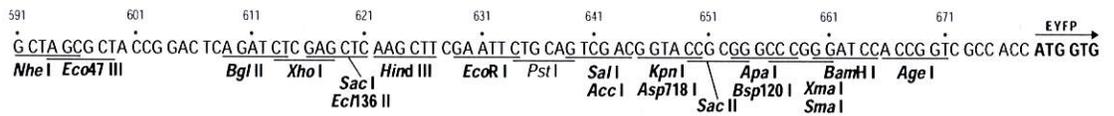
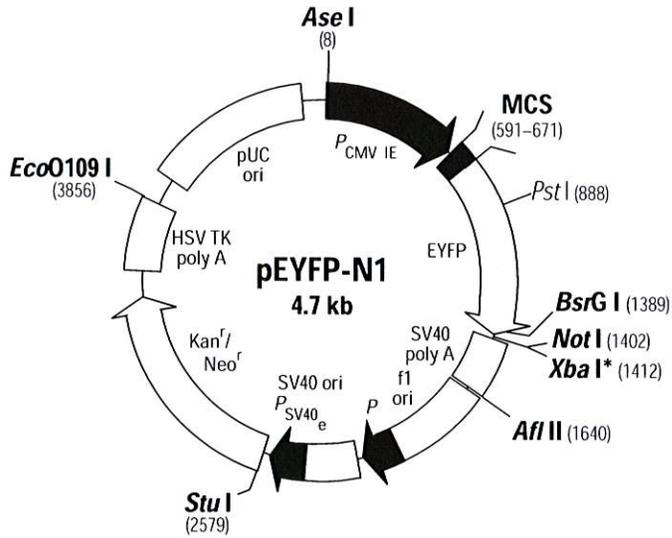
Zufferey R, *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. *Nat. Biotechnol.* **15**, 871-85 (1997).

Zufferey R, *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery, *J Virol.* **72**, 9873-80 (1998).

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Restriction Map and Multiple Cloning Site (MCS) of pEYFP-N1. Unique restriction sites are in bold. The *Xba I* site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest this vector with this enzyme, you will need to transform the vector into a *dam⁻* host and make fresh DNA.

Description:

pEYFP-N1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains four amino acid substitutions previously published as GFP-10C (1). The fluorescence excitation maximum of EYFP is 513 nm, and the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513 nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescence quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence level observed from EYFP is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-N1 is between the immediate early promoter of CMV ($P_{CMV/IE}$) and the EYFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening stop codons. The inserted gene should include an initiating ATG codon. EYFP with N-terminal fusion moieties retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin of replication and a neomycin resistance (*Neo^r*) gene for selection (using G418) in mammalian cells. A bacterial promoter upstream of this cassette (*P*) expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

The recombinant EYFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pEYFP-N1 can also be used simply to express EYFP in a cell line of interest (e.g., as a transfection marker). EGFP, EYFP, and EBFP variants can be used independently or in combination for flow cytometry analysis.

Location of features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
 - Enhancer region: 59–465
 - TATA box: 554–560
 - Transcription start point: 583
 - C→G mutation to remove *Sac*I site: 569
- MCS: 591–671
- Enhanced yellow fluorescent protein (EYFP) gene
 - Kozak consensus translation initiation site: 672–682
 - Start codon (ATG): 679–681; stop codon: 1396–1398
 - Insertion of Val at position 2: 682–684
 - GFP-10C mutations (Ser-65 to Gly: 874–876; Val-68 to Leu: 883–885; Ser-72 to Ala: 895–897; Thr-203 to Tyr: 1288–1290)
 - His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
 - Polyadenylation signals: 1552–1557 & 1581–1586
 - mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104
(Packages the noncoding strand of EYFP.)
- Bacterial promoter for expression of Kan^r gene:
 - 35 region: 2166–2171; –10 region: 2189–2194
 - Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
 - Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
 - 21-bp repeats: 2425–2445, 2446–2466, & 2468–2488
 - Early promoter element: 2501–2507
 - Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
 - Neomycin phosphotransferase coding sequences:
 - Start codon (ATG): 2629–2631; stop codon: 3421–3423
 - G→A mutation to remove *Pst*I site: 2811
 - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 - Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

Propagation in *E. coli*:

- Suitable host strains: DH5 α , HB101, and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Gorman, C. (1985) In *DNA cloning: a practical approach, vol. II*. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

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.

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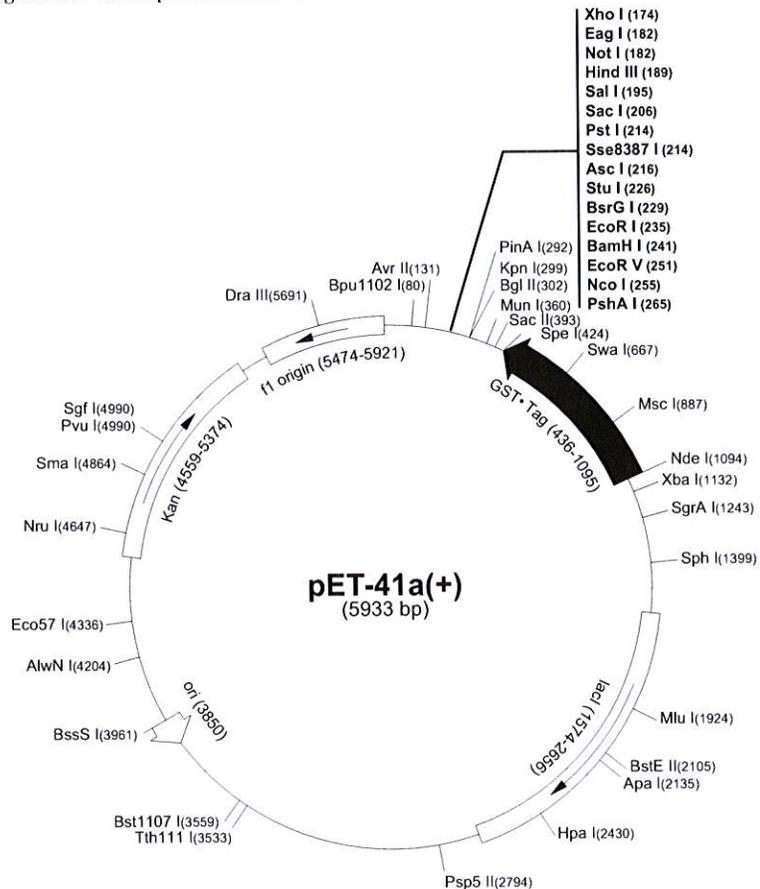
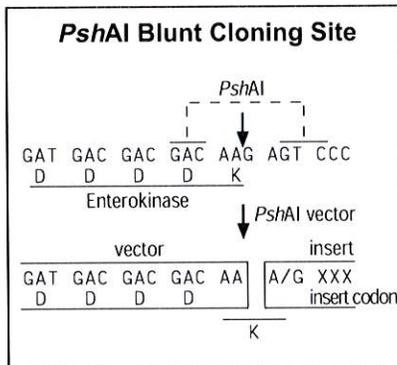
pET-41a-c(+) Vector

	Cat. No.
pET-41a(+) DNA	70556-3
pET-41b(+) DNA	70557-3
pET-41c(+) DNA	70558-3

The pET-41 series is designed for cloning and high-level expression of peptide sequences fused with the 220 aa GST•Tag™ protein. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the T7 terminator primer (cat. no. 69337-3). Vector encoded sequence can be completely removed when cloning into the *PshAI* site (as shown below) and then cleaving the GST fusion protein with Enterokinase.

pET-41a(+) sequence landmarks

T7 promoter	1167-1183
T7 transcription start	1166
GST•Tag coding sequence	436-1095
His•Tag coding sequence	397-414
S•Tag coding sequence	310-354
Multiple cloning sites (<i>PshAI</i> - <i>XhoI</i>)	174-265
His•Tag coding sequence	150-173
T7 terminator	26-72
<i>lacI</i> coding sequence	1574-2656
pBR322 origin	3850
Kan coding sequence	4559-5374
F1 origin	5474-5921



pET-41a(+) cloning/expression regions

pET-41a(+) Restriction Sites

Enzyme	# Sites	Locations				Enzyme	# Sites	Locations				Enzyme	# Sites	Locations			
AccI	2	196	3558			DpnI	23					SacII	1	393			
AcII	73					DraI	2	558	667			Sall	1	195			
AFIII	3	852	1924	3788		DraIII	1	5691				SapI	2	1012	3672		
AluI	25					DrdI	3	3481	3896	5646		Sau3AI	23				
AlwI	12					DsaI	3	255	390	1361		Sau96I	13				
Alw26I	6	1621	2026	2152	2539	EaeI	5	182	885	1232	1364	Scal	2	369	521		
		3429	5006					2598				ScrFI	22				
AlwNI	1	4204				EagI	1	182				SfaNI	24				
ApaI	1	2135				EarI	4	1012	1542	3672	4803	SfiI	5	210	1166	4053	4244
ApaLI	3	1904	3602	4102		Eco47III	2	1329	3042					5910			
ApoI	7	235	331	2199	4603	Eco57I	1	4336				SgfI	1	4990			
		4787	5493	5504		EcoNI	3	1083	1459	4902		SgrAI	1	1243			
AscI	1	216				EcoO109I	4	53	1059	1357	2794	SmaI	1	4864			
AvaI	2	174	4862			EcoRI	1	235				SpeI	1	424			
AvaII	4	597	2476	2794	3073	EcoRII	10	584	1647	1962	2502	SphI	1	1399			
AvrII	1	131						2559	3814	3935	3948	Sse8387I	1	214			
BamHI	1	241				EcoRV	1	251				SspI	2	4915	5483		
BanI	9	295	380	1246	1267	FauI	17				StuI	1	226				
		1381	1844	2563	2693	Fnu4HI	40				StyI	4	57	131	221	255	
		5728				FokI	13				Swal	1	667				
BanII	6	206	1308	1322	2135	HaellI	13				Tail	16					
		4645	5766			HaeIII	24				TaqI	19					
BbsI	3	2070	2409	2906		HgaI	12				TfiI	8	2603	2838	3342	3763	
BbvI	24					Hhal	46							4901	4957	5129	5220
BcgI	4	210	1032	2216	3399	HincII	2	197	2430		Thal	38					
BcgI'	4	176	1066	2250	3365	HindIII	1	189			Tsel	24					
BclI	2	656	1938			HinfI	18				Tsp45I	6	2105	3227	3440	3535	
Bfal	10	70	132	425	1080	HpaI	1	2430						5137	5864		
		1133	2767	2802	4283	HphI	22				Tsp509I	29					
		4590	5842			KpnI	1	299			TspRI	11					
BgIII	1	302				MaeIII	16				Tth111I	1	3533				
BpmI	3	1762	2251	3315		MbolI	15				VspI	6	139	1181	2609	2668	
Bpu10I	2	2894	5007			MluI	1	1924						5189	5378		
Bpu1102I	1	80				MnlI	24				XbaI	1	1132				
BsaAI	2	3540	5691			MscI	1	887			XcmI	3	1780	2296	2314		
BsaBI	3	1197	1207	2985		MseI	31				XhoI	1	174				
BsaHI	5	1247	1268	1382	1881	MsiI	7	1000	1976	2264	2294	XmnI	3	701	3346	5379	
		2564						2775	2970	3361							
BsaJI	12					MspI	28										
BsaWI	9	2	280	292	2243	MspA1I	10	84	344	392	1954						
		2746	2977	3994	4141			2524	2617	3379	3498						
		5125				MunI	1	360									
BsgI	4	810	1775	1975	2948	MwoI	36										
BsiEI	5	185	2709	3704	4128	NarI	4	1247	1268	1382	2564						
		4990				NciI	12										
BsiHKAI	7	175	206	1424	1908	NcoI	1	255									
		2782	3606	4106		NdeI	1	1094									
BsII	28					NgoAIV	2	1234	5792								
BsmI	2	4874	4951			NlaIII	29										
BsmBI	3	2539	3429	5006		NlaIV	22										
BsmFI	5	273	1103	1385	3059	NotI	1	182									
		5906				NruI	1	4647									
Bsp1286I	12					Nsil	2	4840	5106								
BspEI	2	2	2977			NspI	5	856	1399	3133	3425						
BspLU11I	2	852	3788					3792									
BsrI	20					NspV	2	329	695								
BsrBI	5	803	1153	3721	5389	PfiMI	3	321	1506	5253							
		5835				PinAI	1	292									
BsrDI	2	1971	2337			PleI	10	255	1181	1473	1560						
BsrFI	6	292	1234	1243	1610			2356	3682	4167	5222						
		4944	5792			PshAI	1	265									
BsrGI	1	229				Psp1406I	3	1586	3113	5476							
BssHII	2	216	2335			Psp5II	1	2794									
BssSI	1	3961				PstI	1	214									
Bst1107I	1	3559				PvuI	1	4990									
BstEII	1	2105				PvuII	3	2524	2617	3379							
BstXI	3	1726	1855	1978		RcaI	3	1322	4508	5383							
BstYI	8	241	302	1488	2700	RsaI	7	231	297	369	521						
		2980	4429	4440	5239			2071	3594	4825							
CacBI	43					SacI	1	206									
Clal	2	1201	4681														
CviJI	89																
DdeI	11																

Enzymes that do not cut pET41a(+):

AatII	AflII	AhdI	BglII	BsaI	BseRI
BspMI	Bsu36I	FseI	FspI	NheI	PacI
PmeI	PmlI	RsrII	SanDI	SexAI	SfiI
SnaBI	SrfI	SunI	UbaEI		

pET-28a-c(+) Vectors

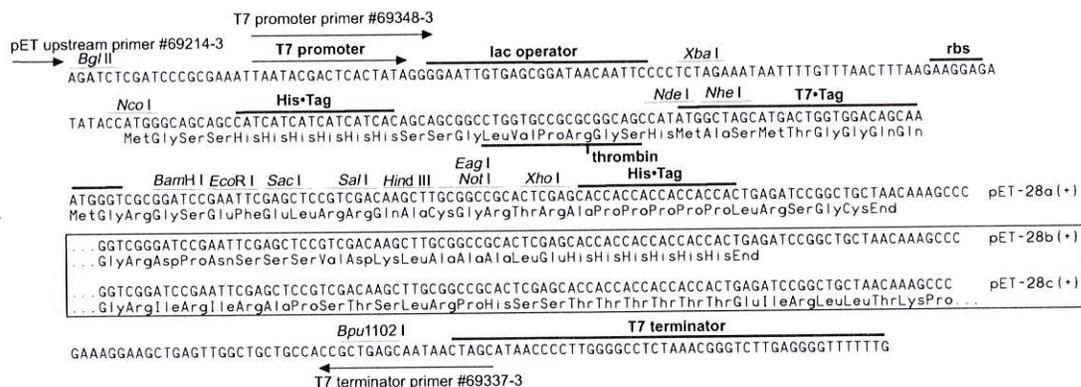
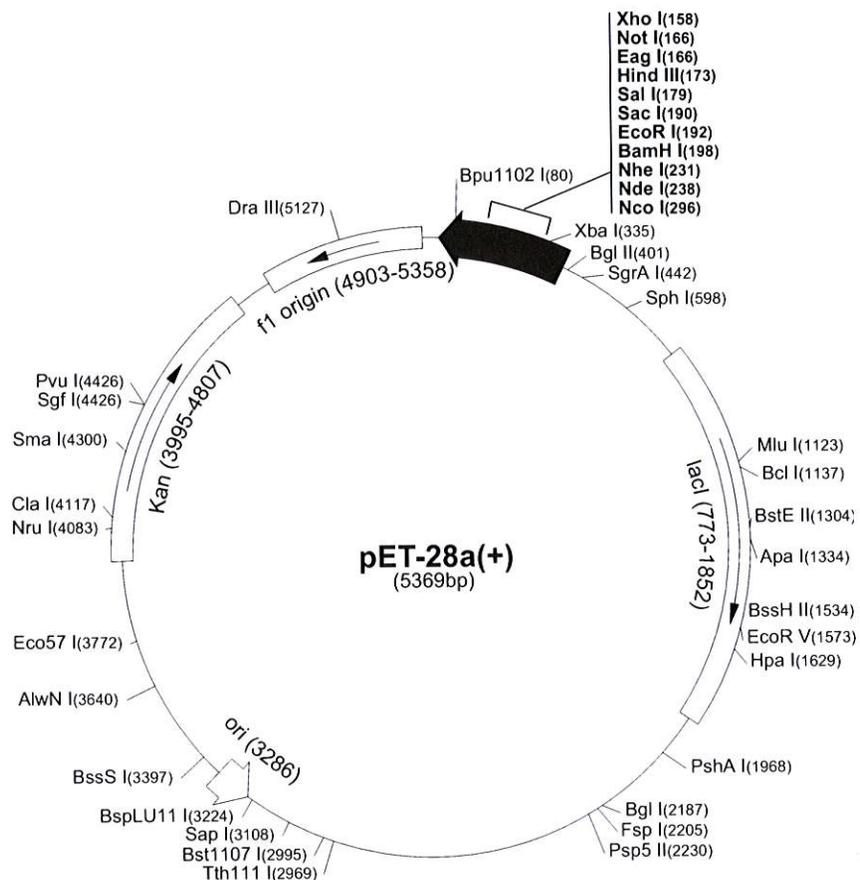
	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

The pET-28a-c(+) vectors carry an N-terminal His•Tag[®]/thrombin/T7•Tag[®] configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites (<i>Bam</i> H I - <i>Xho</i> I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lac</i> I coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.



pET-28a-c(+) cloning/expression region

pET-28a(+) Restriction Sites

Enzyme	# Sites	Locations
AccI	2	180 2994
AccII	7	890 1618 1949 2733 2874 3176 4967
Acil	77	
AflIII	2	1123 3224
AluI	22	
AlwI	13	
Alw21I	7	159 190 623 1107 2218 3042 3542
Alw44I	3	1103 3038 3538
AlwNI	1	3640
Apal	1	1334
ApaBI	1	807
ApoI	6	192 1398 4039 4223 4929 4940
AvaI	2	158 4298
AvaII	5	1675 2051 2139 2230 2509
BamHI	1	198
BanI	9	253 445 466 580 1043 1762 1892 2018 5164
BanII	6	190 507 521 1334 4081 5202
BbsI	4	1269 1608 1982 2342
BbvI	27	
BccI	14	
Bce83I	6	21 1937 2107 3315 3613 3854
BceII	6	642 983 1610 3726 4745 5153
BcgI	9	160 194 228 1415 1449 1949 1983 2801 2835
BclI	1	1137
Bfal	7	70 232 336 2238 3719 4026 5278
BglI	1	2187
BgIII	1	401
BmgI	1	1332
BpmI	4	961 1450 2084 2751
Bpu10I	2	2330 4443
Bpu1102I	1	80
BsaAI	2	2976 5127
BsaBI	3	400 406 2421
BsaHI	5	446 467 581 1080 1763
BsaJI	10	57 296 560 566 1758 2196 3384 4297 4298 4699
BsaWI	7	2 1442 1945 2413 3430 3577 4561
BsaXI	2	1782 5075
Bsbl	2	2940 5034
BscGI	11	
BsgI	3	974 1174 2384
Bsil	1	3397
BsIEI	5	169 1908 3140 3564 4426
BsII	23	
BsmI	2	4310 4387
BsmAI	6	820 1225 1351 1738 2865 4442
BsmBI	3	1738 2865 4442
BsmFI	4	584 2125 2495 5342
BsoFI	48	
Bsp24I	12	
Bsp1286I	12	
BspEI	2	2 2413
BspGI	1	2750
BspLU11I	1	3224
Bsrl	22	
BsRBI	4	356 3157 4825 5271
BsRDI	2	1170 1536
BsRFI	7	433 442 809 2021 2181 4380 5228
BssHII	1	1534
Bst1107I	1	2995

Enzyme	# Sites	Locations
BstEII	1	1304
BstXI	3	925 1054 1177
BstYI	9	132 198 401 687 1899 2416 3865 3876 4675
Cac8I	40	
CjeI	26	
CjePI	30	
Clal	1	4117
CviJI	86	
CviRI	22	
DdeI	11	
Dpnl	21	
DraIII	1	5127
DrdI	3	2917 3332 5082
DrdII	2	846 5132
Dsal	3	296 560 2196
EaeI	4	166 431 563 1797
EagI	1	166
EarI	3	741 3108 4239
Ecil	3	900 3298 3444
Eco47III	3	528 2029 2478
Eco57I	1	3772
EcoNI	2	658 4338
EcoO109I	3	53 556 2230
EcoRI	1	192
EcoRII	10	256 846 1161 1701 1758 3250 3371 3384 4314 4671
EcoRV	1	1573
FauI	17	
FokI	9	1169 1178 2443 2505 2583 2769 2910 4064 4670
FspI	1	2205
GdII	4	166 431 563 1797
HaeI	6	851 2172 3239 3250 3702 4513
HaeII	14	
HaeIII	24	
Hgal	11	
HgiEII	2	721 3810
HhaI	47	
Hin4I	3	1022 4112 4654
HincII	2	181 1629
HindIII	1	173
Hinfl	18	
HpaI	1	1629
HphI	16	
Maell	14	
MaeIII	16	
MbolI	12	
MluI	1	1123
MmeI	7	3439 3623 4068 4262 4624 4633 5104
MnlI	25	
MseI	25	
MslI	6	1175 1463 1493 2211 2406 2797
MspI	29	
MspA1I	9	84 264 1153 1723 1816 2815 2934 3566 3811
MwoI	39	
NarI	4	446 467 581 1763
NciI	12	
NcoI	1	296
NdeI	1	238
NgoAIV	4	433 2021 2181 5228
NheI	1	231
NlaII	26	
NlaIV	22	
NotI	1	166
NruI	1	4083
NsiI	2	4276 4542
NspI	4	598 2569 2861 3228

Enzyme	# Sites	Locations
Pfl1108I	1	2010
PfIMI	2	705 4689
PleI	9	384 672 759 1555 3118 3603 4658 5062 5070
PshAI	1	1968
Psp5II	1	2230
Psp1406I	4	785 2153 2549 4912
PvuI	1	4426
PvuII	3	1723 1816 2815
RcaI	3	521 3944 4819
RsaI	3	1270 3030 4261
SacI	1	190
SalI	1	179
SapI	1	3108
Sau96I	14	
Sau3AI	21	
ScrFI	22	
SfaNI	23	
SfcI	4	369 3489 3680 5346
SgfI	1	4426
SgrAI	1	442
SmaI	1	4300
SphI	1	598
SspI	2	4351 4919
StyI	2	57 296
TaqI	15	
TaqII	6	1031 1249 1922 3126 4680 5031
TfiI	9	1802 2104 2274 2778 3199 4337 4393 4565 4656
Thal	38	
Tsel	27	
Tsp45I	7	1304 2132 2663 2876 2971 4573 5300
Tsp509I	20	
Tth111I	1	2969
Tth111III	8	962 1655 2685 3814 3821 3853 4262 4389
UbaJI	21	
VspI	5	384 1808 1867 4625 4814
XbaI	1	335
XcmI	3	979 1495 1513
XhoI	1	158
Xmnl	2	2782 4815

Enzymes that do not cut pET28a(+):

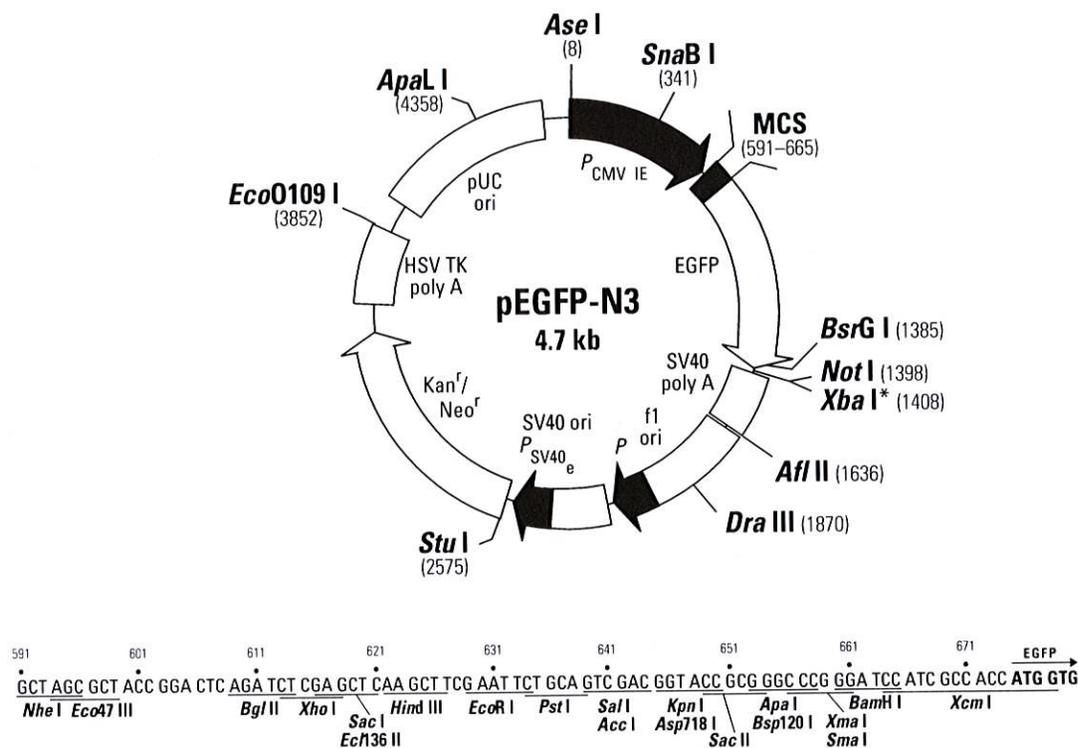
AatII	AflII	AgeI	AscI	AvrII
BaeI	BsaI	BseRI	BspMI	BsrGI
Bsu36I	DraI	Eam1105I	FseI	KpnI
MscI	MunI	NspV	Pacl	PmeI
PmlI	PstI	RleAI	RsrII	SacII
Scal	SexAI	SfiI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	Swal

pEGFP-N3 Vector Information

GenBank Accession #: U57609

PT3054-5

Catalog #6080-1



Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N3 (Unique restriction sites are in bold). The *Not*I site follows the EGFP stop codon. The *Xba*I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-N3 encodes a red-shifted variant of wild-type GFP (1-3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N3 encodes the GFPmut1 variant (4) 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 (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N3 is between the immediate early promoter of CMV ($P_{CMV IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-N3 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N3 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N3 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of Features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
 - Enhancer region: 59–465
 - TATA box: 554–560
 - Transcription start point: 583
 - C→G mutation to remove *Sac* I site: 569
- MCS: 591–665
- Enhanced green fluorescent protein gene
 - Kozak consensus translation initiation site: 668–678
 - Start codon (ATG): 675–677; Stop codon: 1392–1394
 - Insertion of Val at position 2: 678–680
 - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 867–872
 - His-231 to Leu mutation (A→T): 1369
- SV40 early mRNA polyadenylation signal
 - Polyadenylation signals: 1548–1553 & 1577–1582; mRNA 3' ends: 1586 & 1598
- f1 single-strand DNA origin: 1645–2100 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan^r gene:
 - 35 region: 2162–2167; –10 region: 2185–2190
 - Transcription start point: 2197
- SV40 origin of replication: 2441–2576
- SV40 early promoter
 - Enhancer (72-bp tandem repeats): 2274–2345 & 2346–2417
 - 21-bp repeats: 2421–2441, 2442–2462 & 2464–2484
 - Early promoter element: 2497–2503
 - Major transcription start points: 2493, 2531, 2537 & 2542
- Kanamycin/neomycin resistance gene
 - Neomycin phosphotransferase coding sequences: start codon (ATG): 2625–2627; stop codon: 3417–3419
 - G→A mutation to remove *Pst* I site: 2807
 - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3153
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 - Polyadenylation signals: 3655–3660 & 3668–3673
- pUC plasmid replication origin: 4004–4647

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 741–720
- EGFP-C Sequencing Primer (#6478-1): 1328–1349

Propagation in *E. coli*:

- Suitable host strains: DH5 α , HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: \approx 500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Prasher, D. C., *et al.* (1992) *Gene* 111:229–233.
2. Chalfie, M., *et al.* (1994) *Science* 263:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* 341:277–280.
4. Cormack, B., *et al.* (1996) *Gene* 173:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* 6:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125–8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

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.

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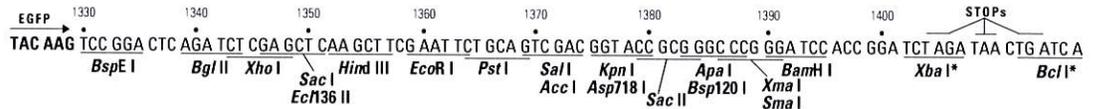
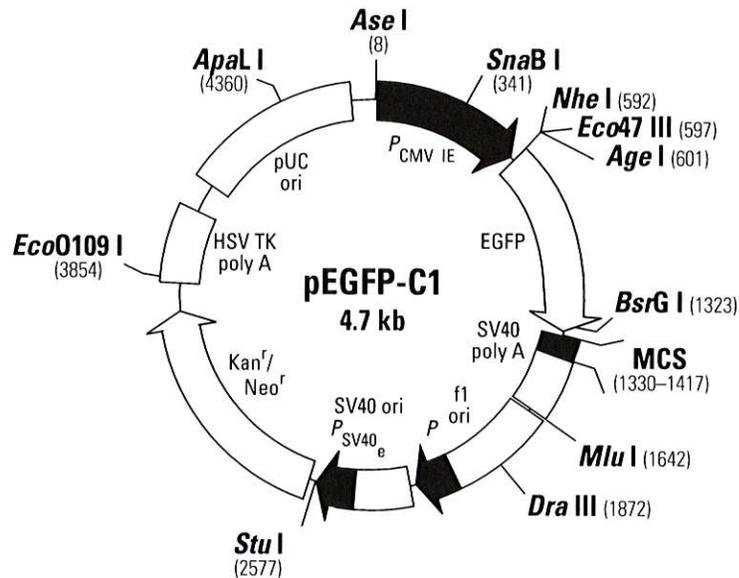
pEGFP-C1 Vector Information

GenBank Accession #: U55763

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PT3028-5

Catalog #6084-1



Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. All restriction sites shown are unique. The *Xba I* and *Bcl I* sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam^r* host and make fresh DNA.

Description

pEGFP-C1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C1 encodes the GFPmut1 variant (4) 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 (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.



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Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR29971; published 03 October 2002)

Use

Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
Enhancer region: 59–465; TATA box: 554–560
Transcription start point: 583
C→G mutation to remove *Sac* I site: 569
- Enhanced green fluorescent protein gene
Kozak consensus translation initiation site: 606–616
Start codon (ATG): 613–615; Stop codon: 1408–1410
Insertion of Val at position 2: 616–618
GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
His-231 to Leu mutation (A→T): 1307
Last amino acid in wild-type GFP: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
–35 region: 2164–2169; –10 region: 2187–2192
Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
21-bp repeats: 2423–2443, 2444–2464, & 2466–2486
Early promoter element: 2499–2505
Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2627–2629; stop codon: 3419–3421
G→A mutation to remove *Pst* I site: 2809
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*

- Suitable host strains: DH5 α , HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: \approx 500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Prasher, D. C., *et al.* (1992) *Gene* 111:229–233.
2. Chalfie, M., *et al.* (1994) *Science* 263:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* 341:277–280.
4. Cormack, B., *et al.* (1996) *Gene* 173:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* 6:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125–8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

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.

Specific EGFP Monoclonal Antibody for Westerns, IP and IC



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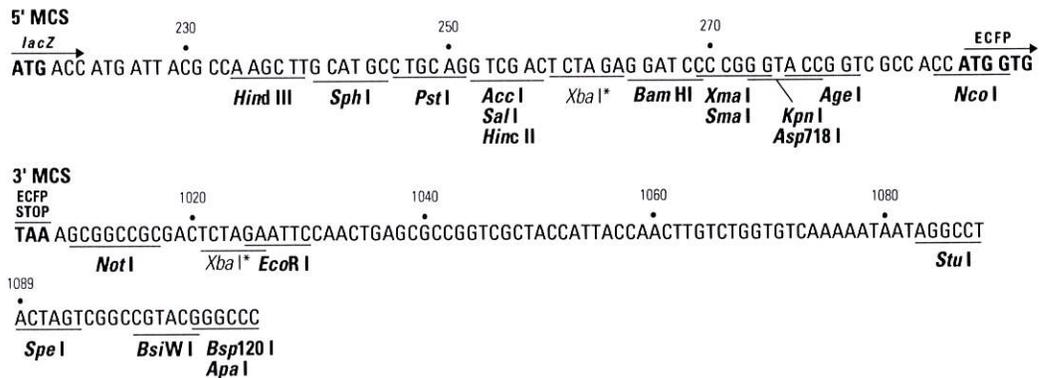
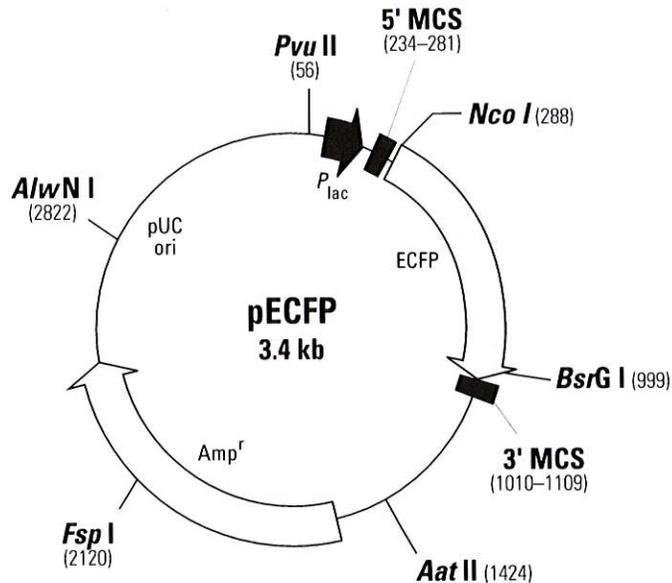
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Restriction map and multiple cloning site (MCS) of pECFP. Unique restriction sites are in bold. The *Xba* I sites in the 5' and 3' MCSs can be used to excise the ECFP gene.

Description:

pECFP encodes an enhanced cyan fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The ECFP gene contains six amino acid substitutions. The Tyr-66 to Trp substitution gives ECFP fluorescence excitation (major peak at 433 nm and a minor peak at 453 nm) and emission (major peak at 475 nm and a minor peak at 501 nm) similar to other cyan emission variants (1–3). The other five substitutions (Phe-64 to Leu; Ser-65 to Thr; Asn-146 to Ile; Met-153 to Thr; and Val-163 to Ala) enhance the brightness and solubility of the protein, primarily due to improved protein folding properties and efficiency of chromophore formation (2, 4, 5).

In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the ECFP mRNA and consequently the expression of ECFP in mammalian and plant cells.

The ECFP gene is flanked at the 5' and 3' ends by the two MCSs of the pUC19 derivative pPD16.43 (8). Thus, the ECFP coding sequence can be easily excised from the vector or amplified by PCR. In *E. coli*, ECFP is expressed from the *lac* promoter as a fusion with several additional amino acids, including the first five amino acids of the *LacZ* protein. Note, however, that if you excise the ECFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., nonfusion) ECFP protein. The pUC19 backbone of ECFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection, respectively in *E. coli*.

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*-ECFP 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 cyan fluorescent protein (ECFP) 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
 - ECFP mutations (Phe-64 to Leu, Ser-65 to Thr, and Tyr-66 to Trp): 481–489; Asn-146 to Ile: 727–729; Met-153 to Thr: 748–750; Val-163 to Ala: 778–780.
 - 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 Locations:

- 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. Heim, R., & Tsien, R. Y. (1996) *Curr. Biol.* **6**:178–182.
2. Mitra, R. D., et al. (1996) *Gene* **173**:13–17.
3. Heim, R. et al. (1994) *Proc. Natl. Acad. Sci. USA* **91**:12501–12504.
4. Cormack, B., et al. (1996) *Gene* **173**:33–38.
5. Yang, T. T., et al. (1996) *Nucleic Acids Res.* **24**:4592–4593.
6. Haas, J., et al. (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. 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.

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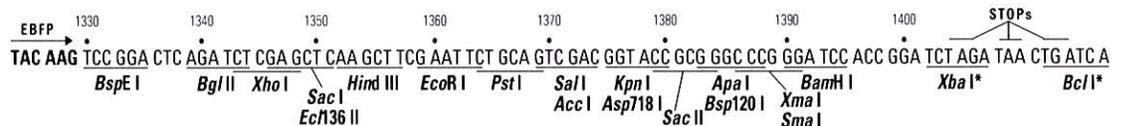
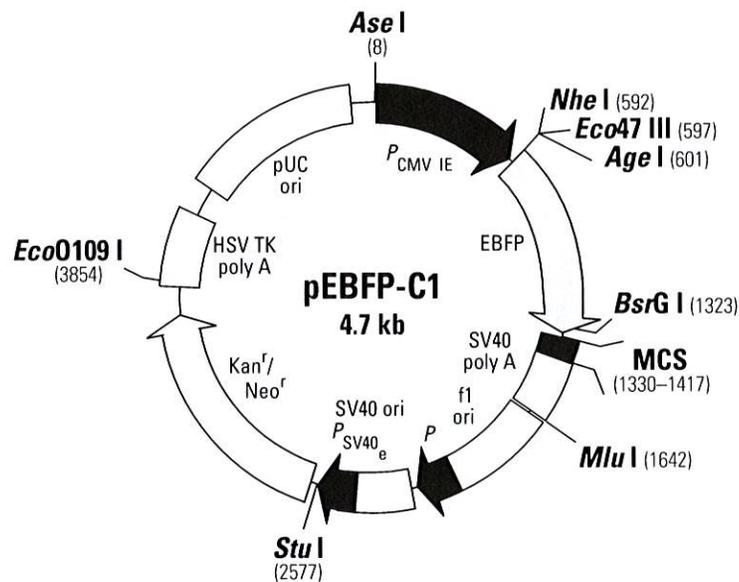
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pEBFP-C1 Vector Information

GenBank Accession #: Submission in progress.

PT3148-5

Catalog #6070-1



Restriction Map and Multiple Cloning Site (MCS) of pEBFP-C1. Unique restriction sites are in bold. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by CLONTECH. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEBFP-C1 carries a blue fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The EBFP gene contains four amino acid substitutions. The Tyr-66 to His substitution gives EBFP fluorescence excitation and emission maxima (380 and 440 nm, respectively) similar to other blue emission variants (1–3). The other three substitutions (Phe-64 to Leu; Ser-65 to Thr; and Tyr-145 to Phe) enhance the brightness and solubility of the protein, primarily due to improved protein-folding properties and efficiency of chromophore formation (1, 4, 5). The E_m of EBFP is $31,000 \text{ cm}^{-1}\text{M}^{-1}$ for 380-nm excitation, leading to a fluorescent signal that is 2–3-fold brighter than other blue variants of GFP and roughly equivalent to wt GFP. In addition, the rate of photobleaching of EBFP is one-half to one-third that of P4-3, a popular predecessor to EBFP (1). EBFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking EBFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the EBFP mRNA and consequently the expression of EBFP in mammalian and plant cells.

The MCS in pEBFP-C1 is between the EBFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EBFP if they are in the same reading frame as EBFP and there are no intervening in-frame stop codons. EBFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (*Neo*^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of *Neo*^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

The recombinant EBFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (8). pEBFP-C1 can also be used simply to express EBFP in a cell line of interest (e.g., as a transfection marker).

Location of features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
Enhancer region: 59–465; TATA box: 554–560; transcription start point: 583
C→G mutation to remove *Sac* I site: 569
- Enhanced blue fluorescent protein gene
Kozak consensus translation initiation site: 606–616
Start codon (ATG): 613–615; stop codon: 1408–1410
Insertion of Val at position 2: 616–618
EBFP mutations (Phe-64 to Leu; Ser-65 to Thr; and Tyr-66 to His): 805–813; Tyr-145 to Phe: 1048–1050
His-231 to Leu mutation (A→T): 1307
Last amino acid in EBFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EBFP.)
- Bacterial promoter for expression of Kan^r gene.
–35 region: 2164–2169; –10 region: 2187–2192
Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
Early promoter element: 2499–2505
Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2627–2629; stop codon: 3419–3421
G→A mutation to remove *Pst* I site: 2809
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:

- Suitable host strains: DH5 α , HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: \approx 500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Heim, R. & Tsien, R. Y. (1996) *Curr. Biol.* **6**:178–182.
2. Mitra, R. D., *et al.* (1996) *Gene* **173**:13–17.
3. Heim, R., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**:12501–12504.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Yang, T. T., *et al.* (1996) *Nucleic Acids Res.* **24**:4592–4593.
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

Notice to Purchaser

Use of CLONTECH's Living Colors® products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Aurora Biosciences Corporation under U.S. Patent Nos. 5,625,048 and 5,777,079 and other pending U.S. and foreign patent applications. In addition, certain CLONTECH products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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All companies and institutions purchasing Living Colors® products will be included in a quarterly report to Aurora Biosciences Corporation, as required by the CLONTECH/Aurora license agreement.

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.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. CLONTECH products may not be resold, modified for resale, or used to manufacture commercial products without written approval of CLONTECH.

pcDNA3.1(+)
pcDNA3.1(-)

Catalog nos. V790-20 and V795-20, respectively

Version I
081401
28-0104



www.invitrogen.com
tech_service@invitrogen.com

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Important Information

Contents

pcDNA3.1 is supplied as follows:

Catalog no.	Contents
V790-20	20 µg pcDNA3.1(+), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0
V795-20	20 µg pcDNA3.1(-), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Product Qualification

Each of the pcDNA3.1 vectors is qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pcDNA3.1(+)	<i>Nhe</i> I	5428
	<i>Pst</i> I	1356, 4072
	<i>Sac</i> I	109, 5319
pcDNA3.1(-)	<i>Nhe</i> I	5427
	<i>Pst</i> I	1363, 4064
	<i>Sac</i> I	169, 5258
pcDNA3.1/CAT	<i>Nhe</i> I	6217
	<i>Pst</i> I	2145, 4072
	<i>Sac</i> I	109, 6008

Purchaser Notification

Introduction

Use of pcDNA3.1 is covered under a number of different licenses as described below.

CMV Promoter

Use of the CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned and licensed by the University of Iowa Research Foundation and may be used **for research purposes only**. Commercial users must obtain a license to these patents directly from the University of Iowa Research Foundation. Inquiries for commercial use should be directed to:

Brenda Akins
University of Iowa Research Foundation (UIRF)
214 Technology Innovation Center
Iowa City, IA 52242
Phone: 319-335-4549

BGH Polyadenylation Signal

The bovine growth hormone (BGH) polyadenylation sequence is licensed under U.S. Patent No. 5,122,458 for research purposes only. "Research purposes" means uses directed to the identification of useful recombinant proteins and the investigation of the recombinant expression of proteins, which uses shall in no event include any of the following:

- a. any use in humans of a CLAIMED DNA or CLAIMED CELL;
- b. any use in human of protein or other substance expressed or made at any stage of its production with the use of a CLAIMED DNA or a CLAIMED CELL;
- c. any use in which a CLAIMED DNA or CLAIMED CELL would be sold or transferred to another party other than Invitrogen, its AFFILIATE, or its SUBLICENSEE;
- d. any use in connection with the expression or production of a product intended for sale or commercial use; or
- e. any use for drug screening or drug development.

Inquiries for commercial use should be directed to:

Bennett Cohen, Ph.D.
Research Corporation Technologies
101 North Wilmot Road, Suite 600
Tucson, AZ 85711-3335
Tel: 1-520-748-4400
Fax: 1-520-748-0025

Methods

Overview

Introduction

pcDNA3.1(+) and pcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 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, pcDNA3.1/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 pcDNA3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA3.1.
 2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 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 pcDNA3.1

Introduction

Diagrams are provided on pages 3-4 to help you design a cloning strategy for ligating your gene of interest into pcDNA3.1. 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', DH5 α TM-T1^R, and TOP10. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*recA*) and endonuclease A-deficient (*endA*).

For your convenience, TOP10F' is available as chemically competent or electrocompetent cells from Invitrogen.

Item	Quantity	Catalog no.
One Shot [®] TOP10F' (chemically competent cells)	21 x 50 μ l	C3030-03
Electrocomp [™] TOP10F'	5 x 80 μ l	C665-55
Ultracomp [™] TOP10F' (chemically competent cells)	5 x 300 μ l	C665-03

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 pcDNA3.1

To propagate and maintain pcDNA3.1, we recommend resuspending the vector in 20 μ l sterile water to make a 1 μ g/ μ l stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *recA*, *endA* *E. coli* strain like TOP10F', DH5 α TM-T1^R, TOP10, or equivalent. Select transformants on LB plates containing 50 to 100 μ g/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

pcDNA3.1(+) and pcDNA3.1(-) are nonfusion vectors. Your insert must contain a Kozak translation initiation sequence and an ATG start codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Please note that other sequences are possible (see references above), but the G or A at position -3 and the G at position +4 are the most critical for function (shown in bold). The ATG initiation codon is shown underlined.

(G/A)NNATGG

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 (TCTAGA).

continued on next page

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(-)

Below is the multiple cloning site for pcDNA3.1(-). 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 pcDNA3.1(-) is available for downloading from our web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(-), please see the **Appendix**, pages 10-11.

```

          ┌──────────────────┐
          │ enhancer region (3' end) │
689  CATTGACGTC AATGGGAGTT TGTTTTGGCA CAAAATCAA CGGGACTTTC CAAAATGTCG

          │ CAAT │
          │       │
749  TAACAAC TCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
          │       │
          │ 3' end of hCMV │
          │               │
          │               │ ────────────────────▶ putative transcriptional start
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

          ┌──────────────────┐
          │ T7 promoter/primer binding site │
          │                               │
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC GGGCCCTCTA GACTCGAGCG
          │                               │
          │ BstX I* EcoR V EcoR I BstX I* BamH I │
          │                               │
929  GCCGCCACTG TGCTGGATAT CTGCAGAATT CCACCACACT GGACTAGTGG ATCCGAGCTC
          │                               │
          │ Asp718 I Kpn I Hind III Afl II Pme I pcDNA3.1/BGH reverse priming site │
          │                               │
989  GGTACCAAGC TTAAGTTTAA ACCGCTGATC AGCCTCGACT GTGCCTTCTA GTTGCCAGCC

1049 ATCTGTTGTT TGCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCA CTCCCCTGTT

          │ BGH poly (A) site │
1109 CCTTTCCTAA TAAAATGAGG AAATTGCATC
  
```

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

continued on next page

Cloning into pcDNA3.1, continued

E. coli **Transformation**

Transform your ligation mixtures into a competent *recA*, *endA* *E. coli* strain (e.g. TOP10F', DH5 α TM-T1^R, TOP10) and select transformants on LB plates containing 50 to 100 μ g/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.



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 μ g 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 μ g/ml ampicillin. Incubate the plate at 37°C overnight.
 - Isolate a single colony and inoculate into 1-2 ml of LB containing 50 μ g/ml ampicillin.
 - Grow the culture to mid-log phase (OD₆₀₀ = 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.
-

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

Plasmid DNA for transfection into eukaryotic cells must be clean and free from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipids decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.[™] MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.[™] MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Methods of Transfection

For established cell lines (e.g. HeLa), please 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). Invitrogen offers the Calcium Phosphate Transfection Kit (Catalog no. K2780-01) and a large selection of reagents for transfection. For more information, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 13).

Positive Control

pcDNA3.1/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).

Creation of Stable Cell Lines

Introduction

The pcDNA3.1(+) and pcDNA3.1(-) 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.

Geneticin® Selective Antibiotic

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

Geneticin® Selection Guidelines

Geneticin® Selective Antibiotic is available from Invitrogen (Catalog no. 10486-025). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 800 µg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (see below). Cells differ in their susceptibility to Geneticin®.

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 up to 3 weeks of growth in selective media.

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your gene of interest from pcDNA3.1, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. We recommend that you test a range of concentrations to ensure that you determine the minimum concentration necessary for your host cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
 2. The next day, substitute culture medium with medium containing varying concentrations of Geneticin® (0, 50, 100, 200, 400, 600, 800 µg/ml Geneticin®).
 3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
 4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Geneticin® that prevents growth within 2-3 weeks after addition of Geneticin®.
-

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Creation of Stable Cell Lines, continued

Possible Sites for Linearization of pcDNA3.1(+)

Prior to transfection, we recommend that you linearize the pcDNA3.1(+) vector. Linearizing pcDNA3.1(+) 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
<i>Bgl</i> II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
<i>Mfe</i> I	161	Upstream of CMV promoter	New England Biolabs
<i>Bst</i> 1107 I	3236	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
<i>Eam</i> 1105 I	4505	Ampicillin gene	AGS*, Fermentas, Takara
<i>Pvu</i> I	4875	Ampicillin gene	Invitrogen, Catalog no. 25420-019
<i>Sca</i> I	4985	Ampicillin gene	Invitrogen, Catalog no. 15436-017
<i>Ssp</i> I	5309	<i>bla</i> promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

Possible Sites for Linearization of pcDNA3.1(-)

The table below lists unique restriction sites that may be used to linearize your pcDNA3.1(-) 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
<i>Bgl</i> II	12	Upstream of CMV promoter	Invitrogen, Catalog no. 15213-028
<i>Mfe</i> I	161	Upstream of CMV promoter	New England Biolabs
<i>Bst</i> 1107 I	3235	End of SV40 polyA	AGS*, Fermentas, Takara, Roche Mol. Biochemicals
<i>Eam</i> 1105 I	4504	Ampicillin gene	AGS*, Fermentas, Takara
<i>Pvu</i> I	4874	Ampicillin gene	Invitrogen, Catalog no. 25420-019
<i>Sca</i> I	4984	Ampicillin gene	Invitrogen, Catalog no. 15436-017
<i>Ssp</i> I	5308	<i>bla</i> promoter	Invitrogen, Catalog no. 15458-011

*Angewandte Gentechnologie Systeme

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Creation of 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.

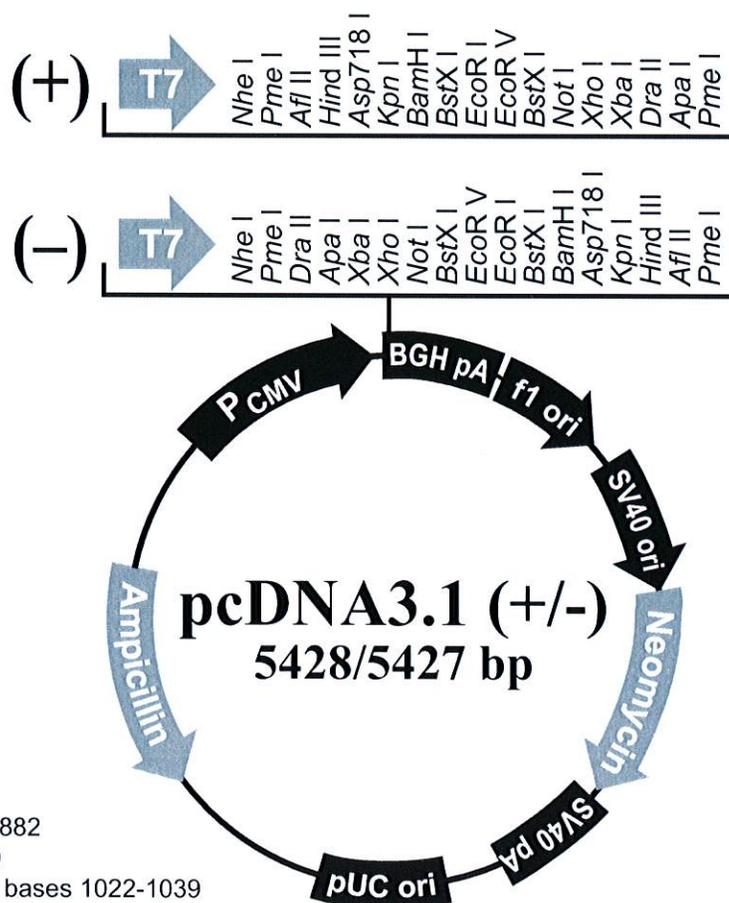
1. Transfect your mammalian host cell line with your pcDNA3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA3.1/CAT plasmid as a positive control.
 2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
 3. 48 hours after transfection, split the cells into fresh medium containing Geneticin[®] at the pre-determined 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

pcDNA3.1 Vectors

Map of pcDNA3.1(+) and pcDNA3.1(-)

The figure below summarizes the features of the pcDNA3.1(+) and pcDNA3.1(-) vectors. The complete sequences for pcDNA3.1(+) and pcDNA3.1(-) are available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (see page 13). Details of the multiple cloning sites are shown on page 3 for pcDNA3.1(+) and page 4 for pcDNA3.1(-).



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

pcDNA3.1 Vectors, continued

Features of pcDNA3.1(+) and pcDNA3.1(-)

pcDNA3.1(+) (5428 bp) and pcDNA3.1(-) (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 <i>et al.</i> , 1989; Boshart <i>et al.</i> , 1985; Nelson <i>et al.</i> , 1987)
T7 promoter/priming site	Allows for <i>in vitro</i> 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 <i>E. coli</i>
Ampicillin resistance gene (β -lactamase)	Selection of vector in <i>E. coli</i>

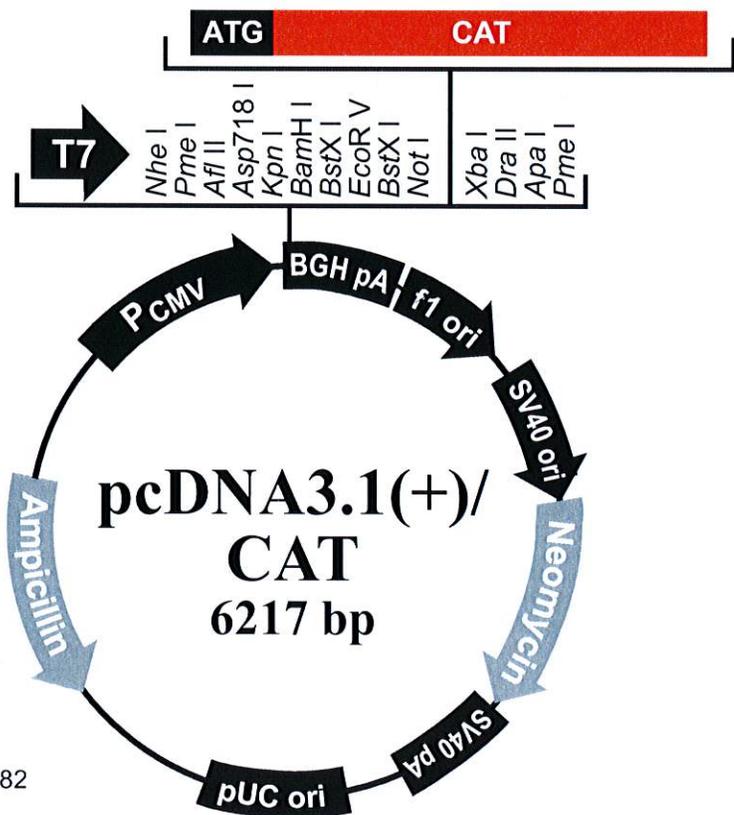
pcDNA3.1/CAT

Description

pcDNA3.1/CAT is a 6217 bp control vector containing the gene for CAT. It was constructed by digesting pcDNA3.1(+) with *Xho* I and *Xba* I and treating with Klenow. An 800 bp *Hind* III fragment containing the CAT gene was treated with Klenow and then ligated into pcDNA3.1(+).

Map of Control Vector

The figure below summarizes the features of the pcDNA3.1/CAT vector. The complete nucleotide sequence for pcDNA3.1/CAT is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (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 Service

World Wide Web



Visit the [Invitrogen Web Resource](#) using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

<http://www.invitrogen.com>

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact us

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

United States Headquarters:

Invitrogen Corporation
1600 Faraday Avenue
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tech_service@invitrogen.com

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Tel: 81 3 3663 7972
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Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, please visit our web site (www.invitrogen.com) and follow the instructions below.

1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
 2. Follow instructions on the page and fill out all the required fields.
 3. To request additional MSDSs, click the 'Add Another' button.
 4. All requests will be faxed unless another method is selected.
 5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.
-

continued on next page

Technical Service, continued

Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
Voice: 1-760-602-8700

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to the specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

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Product Information

p3XFLAG-CMV™-10 EXPRESSION VECTOR

Product No. **E 4401**

Store at 0 to -20 °C

Product Description

p3XFLAG-CMV™-10 expression vector is a 6.3 kb derivative of pCMV5¹ used to establish transient or stable intracellular expression of N-terminal 3XFLAG fusion proteins in mammalian cells. The vector encodes three adjacent FLAG® epitopes (Asp-Tyr-Lys-Xaa-Xaa-Asp) upstream of the multiple cloning region. This results in increased detection sensitivity using ANTI-FLAG® M2 antibody.² The third FLAG epitope includes the enterokinase recognition sequence, allowing cleavage of the 3XFLAG peptide from the purified fusion protein.

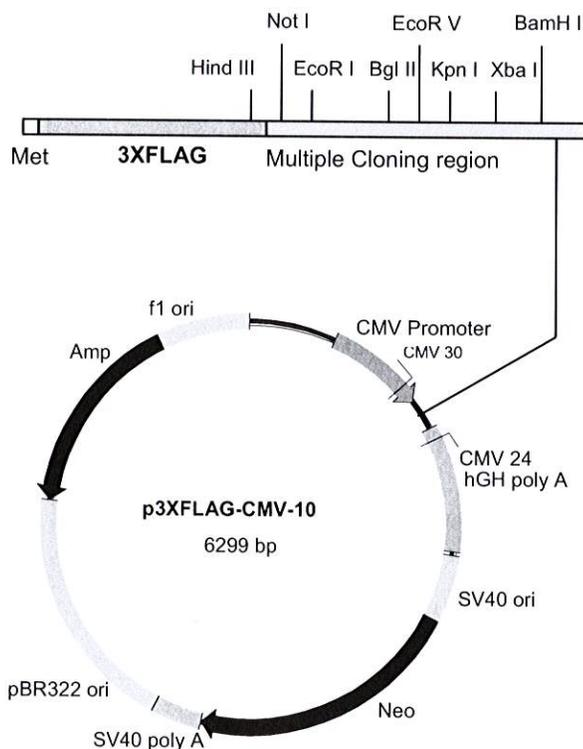
The promoter-regulatory region of the human cytomegalovirus³ drives transcription of FLAG-fusion constructs. The aminoglycoside phosphotransferase II gene (Neo) confers resistance to aminoglycosides such as G 418,⁴ allowing for selection of stable transfectants.

p3XFLAG-CMV-10 expression vector is a shuttle vector for *E. coli* and mammalian cells. Efficiency of replication is optimal when using an SV40 T antigen-expressing host, such as COS cells.

p3XFLAG-CMV-10 expression vector is supplied in 10 mM Tris, 1 mM EDTA, pH 8.0.

References

1. Andersson, S., *et al.*, J. Biol. Chem., **264**, 8222-8229 (1989)
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3. Thomsen, D.R., *et al.*, Proc. Natl. Acad. Sci. USA, **81**, 659-663 (1984)
4. Jimenez, A. and Davies, J., Nature, **287**, 869-871 (1980)

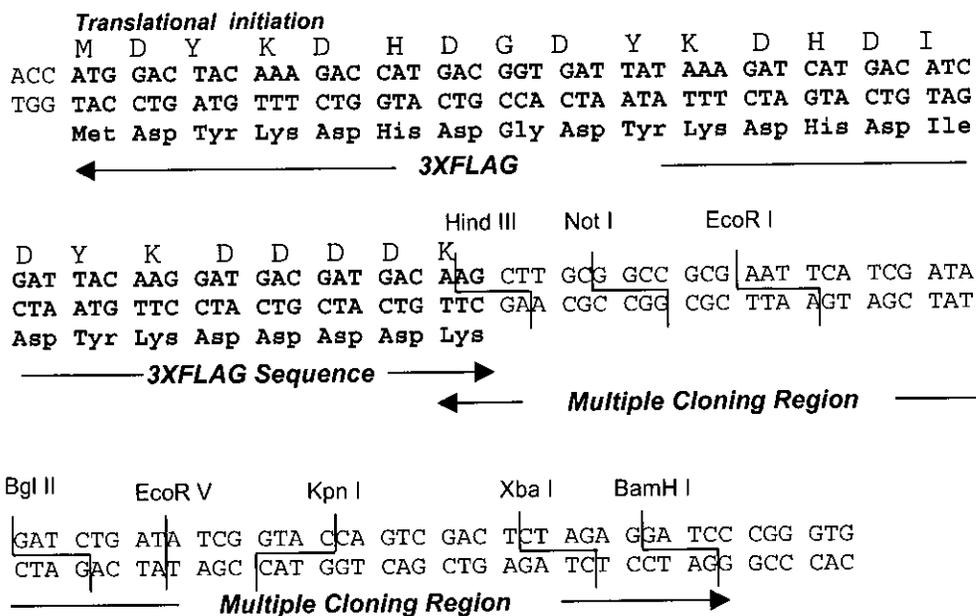


p3XFLAG-CMV-10 Features

Feature	Map Position
CMV promoter	166-916
CMV 30 sequencing primer	825-854
Translational initiation	928-930
3XFLAG sequence	931-996
Multiple cloning region	994-1056
hGH poly A	1061-1680
CMV 24 sequencing primer	1118-1141
SV40 ori	1699-2037
Neo	2073-2864
SV40 poly A	3511-3609
pBR322 ori	4528-4647
Ampicillin resistance	4824-5684
f1 ori	5847-6299

Nucleotide Sequence of the Multiple Cloning Region of the p3XFLAG-CMV-10 Expression Vector

Sequence range: 925 to 1061



07/03

These products and/or their use are covered by one or more of the following patents: US 5,011,912, US 4,703,004, US 4,782,137, US 4,851,341, EP 150126, EP 335899, JP 1983150, JP 2665359, CA 1307752. Use of these products are subject to the terms of a license provided in the product packaging, a copy of which will be provided upon request. FLAG[®] and ANTI-FLAG[®] registered trademarks of Sigma-Aldrich Biotechnology LP. The product designations of pFLAG[™], p3XFLAG[™], pFLAG-1[™], pFLAG-2[™], pFLAGSHIFT[™], pFLAG-CTS[™], pFLAG-ATS[™], pFLAG-MAC[™], pFLAG-CMV[™], YEpFLAG[™], and FLAG-BAP[™] are trademarks of Sigma-Aldrich Biotechnology LP.

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A. Vector License: You may use the enclosed vector to transform cells to produce proteins containing the amino acid sequence DYKDDDDK for research purposes provided, however, such research purposes do not include binding an unlicensed antibody to any portion of this amino acid sequence nor using such proteins for the preparation of antibodies having an affinity for any portion of this amino acid sequence.

B. Antibody License: You may only use the enclosed antibody for research purposes to perform a method of producing a protein in which the protein is expressed in a host cell and purified by use of the antibody in accordance with a claim in one of the above patents in force in a country where the use actually occurs so long as: (1) you perform such method with a DNA expression vector licensed from Sigma-Aldrich Co.; and (2) you do not bind (or allow others to bind) an unlicensed antibody to any DYKDDDDK epitope of any fusion protein that is produced by use of the method.

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If the terms and conditions of this License Agreement are acceptable to you, then you may open the vessel(s) containing the vector and/or antibody and, through such act of opening a vessel, will have shown your acceptance to these terms and conditions.

If the terms and conditions of this License Agreement are not acceptable to you, then please return the vessel(s) unopened to Sigma-Aldrich Co. for a complete refund of your payment.

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Public Health Agency of Canada
Centre for Emergency Preparedness and Response

Agence de la santé publique du Canada
Centre de mesures et d'interventions d'urgence

Permit no.-Permis no.

Permit to import human pathogen(s)

**Permis d'importation d'agent(s)
anthropopathogène(s)**

P- 17116

Under the authority of the Human Pathogens Importation Regulations.

Sous le régime du Règlement sur l'importation des agents anthropopathogènes.

Importer-Name, address and postal code - Importateur-Nom, adresse et code postal

Facsimile-Télécopieur

Telephone no. - No. de téléphone

University of Western Ontario
Department of Microbiology and Immunology
1151 Richmond Street
London, ON N6A 5C1

519-661-3499

519-661-3438

Attn: Dr. Stephen Barr

Supplier-Name and address - Fournisseur-Nom et adresse

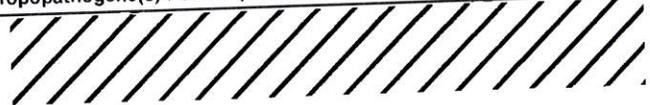
Name(s) of Port(s) of Entry- To Clear Customs at Port(s) of entry
Nom(s) de(s) point(s) d'entrée -Dédouanement au(x) point(s) d'entrée

NIH Nonhuman Primate Regent Resource
Beth Israel Deaconess Medical Center
Division of Viral Pathogenesis, E/CLS 1038
330 Brookline Avenue
Boston, MA 02215, USA

Various ports

Description of Pathogen(s)-For the importation of- Description de(s) agent(s) anthropopathogène(s)-Pour l'importation

Cynomolgus T cell line(HSC-F) from Macaca fascicularis immortalized by transformation with Herpes virus saimiri*.



*Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for importation -
*Les agents anthropopathogènes indiqués sur ce permis doivent aussi être accompagnés d'un permis d'importation de l'ACIA.

On the following terms and conditions as marked:-Selon les conditions indiquées:

- | | |
|--|--|
| <p>1. Work involving any of the imported material shall be limited to <i>in vitro</i> laboratory studies.</p> | <p><input checked="" type="checkbox"/> Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire <i>in vitro</i>.</p> |
| <p>2. Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.</p> | <p><input checked="" type="checkbox"/> Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.</p> |
| <p>3. All animals exposed to infection by any of the imported material shall be so exposed and held only in isolated insect-and rodent-proof facilities.</p> | <p><input type="checkbox"/> Les animaux exposés à l'infection par la matière importée doivent y être exposés et être gardés uniquement dans des installations isolées à l'abri des insectes et des rongeurs.</p> |
| <p>4. All equipment, animal pens, cages, bedding, waste and other articles under the importer's control, that come in direct or indirect contact with any of the imported material, shall be sterilized by autoclaving or incinerated.</p> | <p><input checked="" type="checkbox"/> L'équipement, les enclos pour animaux, les cages, les litières, les déchets et tout autre article sous la responsabilité de l'importateur qui viennent en contact direct ou indirect avec la matière importée doivent être stérilisés par autoclavage ou incinérés.</p> |
| <p>5. Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.</p> | <p><input checked="" type="checkbox"/> Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.</p> |
| <p>6. No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.</p> | <p><input checked="" type="checkbox"/> La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis. AUCUNE AGENT ANTHROPOPATHOGENE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.</p> |
| <p>7. On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.</p> | <p><input checked="" type="checkbox"/> Au terme des travaux de l'importateur auxquels a servi l'agent anthropopathogène importé, celui-ci et tous ses dérivés doivent être détruits.</p> |
| <p>8. Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.</p> | <p><input checked="" type="checkbox"/> On peut accomplir l'isolation, l'identification primaire, et/ou la manipulation au niveau de confinement 2 (exigences physiques) en utilisant les exigences opérationnelles de niveau de confinement 3.</p> |
| <p>9. NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.</p> | <p><input type="checkbox"/> AUCUNE MATIÈRE IMPORTÉE NE PEUT ÊTRE TRANSPORTÉE, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MISE EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.</p> |
| <p>10. The Director must approve all new work with the imported material involving construction of recombinants that requires an increase of containment from level 2.</p> | <p><input type="checkbox"/> Tous nouveaux travaux de manipulation génétique (recombiné) avec la matière importée qui demandera que le niveau 2 de confinement soit augmenté exigera l'approbation du Directeur.</p> |
| <p>11. No culturing of Risk Group 3 or 4 pathogens shall be done.</p> | <p><input checked="" type="checkbox"/> Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise.</p> |

12. This permit is valid only for:
Le présent permis n'est valide que pour:

- a) a single entry into Canada or
une seule entrée au Canada ou

and ending on

immortalized by transformation with Herpes virus saimiri*.

*Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for Importation -

*Les agents anthropopathogènes indiqués sur ce permis doivent aussi être accompagnés d'un permis d'importation de l'ACIA.

On the following terms and conditions as marked:-Selon les conditions indiquées:

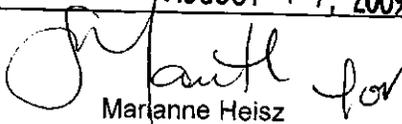
- | | |
|--|--|
| <p>1. Work involving any of the imported material shall be limited to <i>in vitro</i> laboratory studies.</p> | <p><input checked="" type="checkbox"/> Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire <i>in vitro</i>.</p> |
| <p>2. Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.</p> | <p><input checked="" type="checkbox"/> Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.</p> |
| <p>3. All animals exposed to infection by any of the imported material shall be so exposed and held only in isolated insect-and rodent-proof facilities.</p> | <p><input type="checkbox"/> Les animaux exposés à l'infection par la matière importée doivent y être exposés et être gardés uniquement dans des installations isolées à l'abri des insectes et des rongeurs.</p> |
| <p>4. All equipment, animal pens, cages, bedding, waste and other articles under the importer's control, that come in direct or indirect contact with any of the imported material, shall be sterilized by autoclaving or incinerated.</p> | <p><input checked="" type="checkbox"/> L'équipement, les enclos pour animaux, les cages, les litières, les déchets et tout autre article sous la responsabilité de l'importateur qui viennent en contact direct ou indirect avec la matière importée doivent être stérilisés par autoclavage ou incinérés.</p> |
| <p>5. Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.</p> | <p><input checked="" type="checkbox"/> Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.</p> |
| <p>6. No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.</p> | <p><input checked="" type="checkbox"/> La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis. AUCUNE AGENT ANTHROPOPATHOGÈNE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.</p> |
| <p>7. On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.</p> | <p><input checked="" type="checkbox"/> Au terme des travaux de l'importateur auxquels a servi l'agent anthropopathogène importé, celui-ci et tous ses dérivés doivent être détruits.</p> |
| <p>8. Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.</p> | <p><input checked="" type="checkbox"/> On peut accomplir l'isolation, l'identification primaire, et/ou la manipulation au niveau de confinement 2 (exigences physiques) en utilisant les exigences opérationnelles de niveau de confinement 3.</p> |
| <p>9. NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.</p> | <p><input type="checkbox"/> AUCUNE MATIÈRE IMPORTÉE NE PEUT ÊTRE TRANSPORTÉE, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MISE EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.</p> |
| <p>10. The Director must approve all new work with the imported material involving construction of recombinants that requires an increase of containment from level 2.</p> | <p><input type="checkbox"/> Tous nouveaux travaux de manipulation génétique (recombiné) avec la matière importée qui demandera que le niveau 2 de confinement soit augmenté exigera l'approbation du Directeur.</p> |
| <p>11. No culturing of Risk Group 3 or 4 pathogens shall be done.</p> | <p><input checked="" type="checkbox"/> Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise.</p> |

12. This permit is valid only for: a) a single entry into Canada or
Le présent permis n'est valide que pour: une seule entrée au Canada ou
- b) importations at intervals of during the period beginning on and ending on
les importations effectuées à intervalles de au cours de la période commençant le et se terminant le

AUGUST 17, 2009

AUGUST 31, 2010

Authorization-Signature of Director
Autorisation-Signature du Directeur


Marianne Heisz

Date AUGUST 17, 2009

Note: Transporting and otherwise dealing with imported material are subject to federal, provincial and municipal laws (if any), to the extent that, those laws apply in respect of that material.

Nota: Les opérations relatives à la matière importée, y compris le transport, sont assujetties aux lois fédérales, provinciales et aux règlements municipaux applicables.

Canada

Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-03559-4
ORIGINAL
2009/08/04
year/mo/day
année/mois/jour

IMPORT PERMIT

PERMIS D'IMPORTATION

Page 1 of de 3

THIS PERMIT IS ISSUED PURSUANT TO/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A:

THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE DEPART. OF MICROBIOLOGY AND IMMUNOLOGY DENTAL SCIENCES BUILDING LONDON, ONTARIO N6A5C1 Applicant Name: DR. STEPHEN BARR Phone: 519-661-3438 Fax: 519-661-3499 Email: STEPHEN.BARR@BARR@UWO.CA		Exporter/Exportateur NIH NONHUMAN PRIMATE REAGENT RESOURCES 330 BROOKLINE AVENUE BOSTON, MASSACHUSETTS UNITED STATES 02215 Contact: Keith A. Reimann Phone: 617-735-4476 Fax: 617-735-4527	
Quarantine/Destination/Quarantaine		Producer/Producteur	
Valid/Valide	from/du 2009/08/04 year/month/day année/mois/jour	to/au 2010/08/31 year/month/day année/mois/jour	Country of Origin/ Pays d'Origine UNITED STATES (MASSACHUSETTS)
For the entry of/ Pour l'entrée de:		Single shipment/Chargement simple <input checked="" type="checkbox"/> Multiple shipments/Chargements multiples	
Place of entry into Canada/Lieu d'entrée au Canada:			
FOR THE IMPORTATION OF:/POUR L'IMPORTATION DE: (Description of things(s)/Description de la ou des choses) 1. Product Description: CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRSNFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI. (TO BE USED IN VITRO ONLY IN DENTAL SCIENCES BLDG ROOM 6006, DEPT. OF MICROBIOLOGY & IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON) Proposed End Use: "In Vitro" Scientific Name: Biocontainment Level: 2			
A PERSON WHO IMPORTS A THING UNDER THIS PERMIT SHALL COMPLY WITH ALL THE CONDITIONS SET OUT HEREIN/TOUTE PERSONNE QUI IMPORTE UNE CHOSE EN VERTU DE CE PERMIS DEVRA RESPECTER TOUTES LES CONDITIONS DÉCRITES CI-DESSOUS			

Selected Conditions / Conditions Choies

CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRSNFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI.

(TO BE USED IN VITRO ONLY IN DENTAL SCIENCES BLDG ROOM 6006, DEPT. OF MICROBIOLOGY & IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON)

1. The original or a copy of the signed original of this permit and any other necessary import / export documentation pertaining to the shipment of animal(s) or thing(s) must be provided for inspection at the first port of entry or to a Canadian Food Inspection Agency Import Service Center.

2. The conditions in this permit can only be changed or amended by a CFIA inspector. Any change to the permit by an unauthorized person will render the permit invalid.



Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-03559-4
ORIGINAL
2009/08/04
year/mo/day
année/mois/jour

IMPORT PERMIT

PERMIS D'IMPORTATION

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THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur	Exporter/Exportateur
UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE	NIH NONHUMAN PRIMATE REAGENT RESOURCES
DEPART. OF MICROBIOLOGY AND IMMUNOLOGY DENTAL SCIENCES BUILDING LONDON, ONTARIO N6ASC1	330 BROOKLINE AVENUE BOSTON, MASSACHUSETTS UNITED STATES 02215
Applicant Name: DR. STEPHEN BARR Phone: 519-661-3438 Fax: 519-661-3499 Email: STEPHEN.BARR@BARR@UWO.CA	Contact: Keith A. Reimann Phone: 617-735-4476 Fax: 617-735-4527

Selected Conditions / Conditions Choies (Continued/Suite)

3. The imported material must be packaged in appropriate shipping containers to prevent accidental spillage of contents during shipping. Importers should be aware of their obligations under Transport Canada's regulations concerning transportation of dangerous goods.
4. All infectious material must be handled in appropriate animal pathogen containment level 2 facilities as described in Containment Standards for Veterinary Facilities, 1996, AAFC publication no. 1921.
5. The material authorized for importation by this permit is to be used in in vitro studies ONLY and must not be introduced into laboratory, domestic or wild animals (including birds or fish) unless written authorization is obtained from the Canadian Food Inspection Agency.
6. The animal(s) or thing(s) imported under this permit must NEVER be removed from the premises of destination listed on this permit, even after the animals have been released from their post-import quarantine, unless written authorization is obtained from the Canadian Food Inspection Agency.
7. Upon completion of the tests or experiments, the imported material as described on this permit and any derivatives thereof must be autoclaved, incinerated or alternatively disposed of in a manner approved by an inspector of the Canadian Food Inspection Agency.
8. Records pertaining to the imported product's use, storage and disposal must be maintained for two (2) years following importation. These records must be made available for inspection by the Canadian Food Inspection Agency upon request.
9. The importer is responsible for all costs incurred or associated with any testing or treatment of the animal(s) or thing(s) that may be required under the import permit or under the authority of the Health of Animals Act or the Health of Animals Regulations. The importer shall pay all fees for services required in respect of the importation under the National Animal Health Program Cost Recovery Fees Regulations in place at the time of importation.
10. Consideration of an application necessary for issuance of a permit to import the described animal or thing is subject to Class 1 fees.
11. The issuance of this permit does not relieve the owner or the importer of the obligation to comply with any other relevant federal, provincial or municipal legislation or requirement.
12. Failure to comply with the conditions contained in this permit or with the provisions of the Health of Animals Act and Regulations may result in the cancellation of this permit and will result in the forfeiture to the Crown of the imported thing(s) or in the removal of the thing(s) from Canada, all without compensation to, and at the expense of the importer. The importer(s) are responsible for the imported thing(s), their freedom from extraneous disease, active or latent, and genetic or other defects. The importer, his heirs, executors, successors and assigns release and discharges Her Majesty the Queen in right of Canada and the CFIA of and from all claims and demands, damages, actions or causes of action arising or to arise by reason of the importation of the thing(s) and agrees to indemnify and save harmless Her Majesty the Queen in right of Canada and the CFIA from and against all actions, damages, claims and demands which may be brought in respect of or arising out of the importation of such thing(s), any contamination with extraneous disease or other defects.



Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
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Importer/Importateur

UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE

DEPART. OF MICROBIOLOGY AND IMMUNOLOGY
DENTAL SCIENCES BUILDING
LONDON, ONTARIO
N6A5C1

Applicant Name: DR. STEPHEN BARR

Phone: 519-661-3438 Fax: 519-661-3499

Email: STEPHEN.BARR@BARR@UWO.CA

Exporter/Exportateur

NIH NONHUMAN PRIMATE REAGENT RESOURCES

330 BROOKLINE AVENUE
BOSTON, MASSACHUSETTS
UNITED STATES 02215

Contact: Keith A. Reimann

Phone: 617-735-4476 Fax: 617-735-4527

Selected Conditions / Conditions Choisies (Continued/Suite)

13. This permit is conditional upon a permit being obtained under the Human Pathogens Importation Regulations to import the pathogenic material and upon that import permit being produced and valid when the above pathogenic material is presented to an inspector for inspection at the time of importation.

Additional Conditions Additionnelles

CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRANSFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI.

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1. LEVEL 2 PHYSICAL CONTAINMENT AND LEVEL 3 OPERATIONAL PRACTICES ARE REQUIRED.
2. NO CULTURING OF CONTAINMENT LEVEL 3 OR 4 PATHOGENS SHALL BE DONE.

Cynthia Labrie
Authorized By:/Approuvé par:
CINTHIA LABRIE

For the Minister of Agriculture and Agri-Food
Pour le ministre d'agriculture et agroalimentaire

The information is required by (for) the Canadian Food Inspection Agency for the purpose of verifying import products. Information may be accessible or protected as required under the provisions of the Access to Information Act.

