

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 and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

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:	Dale W Laird
DEPARTMENT:	Anatomy and Cell Biology
ADDRESS:	DSB 00077
PHONE NUMBER:	519 661-2111 x86827
EMERGENCY PHONE NUMBER(S):	519 673-3343
EMAIL:	dale.laird@schulich.uwo.ca

Location of experimental work to be carried out :

Building :	Dental Science Building	Room(s):	00077
Building :	Dental Science Building	Room(s):	00066
Building :	Dental Science Building	Room(s):	00015, 00017

***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: **CIHR, CBCF, CRC**

GRANT TITLE(S): **Cx26 and Panx1 as breast tumor suppressors: potential therapeutic targets CBCF**
Cx43 mutations linked to human disease CIHR
Comparative analysis of the life cycle and function of connexins & pannexins CIHR
Functional role of connexins in ... CIHR

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Dale W Laird	dale.laird@schulich.uwo.ca	1997
Qing (Cindy) Shao	cindy.shao@schulich.uwo.ca	1998, 2002,

Silvia Penuela	silvia.penuela@schulich.uwo.ca	Oct. 2005
Xiang-Qun Gong	xiang-qun.gong@schulich.uwo.ca	May 2003
Tao Huang	tao.huang@schulich.uwo.ca	May 2011
John Kelly	john.kelly@schulich.uwo.ca	Feb 2012
Kevin Barr	kevin.barr@schulich.uwo.ca	2004
Michael Stewart	michael.stewart@schulich.uwo.ca	Aug 2009
Amy Berger	amy.berger@schulich.uwo.ca	June 2009
Mark (Jake) Ableser	mableser@gmail.com	Sept 2011
Jamie Simek	jamie.simek@schulich.uwo.ca	Oct 2006
Shreya Podder	spodder2@uwo.ca	Oct 2010
Wesley Lai	wlai43@uwo.ca	May 2011

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.

Cell Lines: All cell lines including viral packaging cells are cultured and passaged in laminar flow hoods that are certified annually for level 2 containment. For long term storage cells are initially frozen in a biohazard labeled -80oC freezer prior to being transferred to a biohazard labeled liquid nitrogen tanks one or two days after freezing. Unwanted cells are bleached and autoclaved prior to disposal. Cells expressing connexins, pannexins or site-directed mutants of these molecules as well as cells ectopically expressing cDNAs are frequently used for Western blots, immunofluorescent or other biochemical analysis.

cDNA constructs: All cDNA constructs(connexins and pannexins) are stored in a -20oC freezer and viral particles are stored in a biohazard labeled -80oC freezer. Reference cell lines or primary cells are transfected or infected with cDNAs to assess the the biochemical and functional properties of connexins and pannexins. Stably infected or transfected cells are cultured and passaged under level 2 containment. Unwanted transfected or infected cell lines are killed by bleaching , autoclaving, and disposal as biohazardous labeled waste.

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

Most cells in the human body need to consistently talk to each other and exchange information. Cell to cell communication can be mediated by connexin channels called gap junctions that directly allow the exchange of signals. In other cases, information encoded in small molecules are released through specialized channels only to be recaptured by receptors or channels that reside on the surface of neighboring cells. In the present study we are focusing on a new class of channel forming proteins called pannexins that allow small molecules carrying important signals to enter and leave the cell. Already these unique channels have been demonstrated to be important in sending find me signals to clear dead or dying cells. In disease, pannexin channels have been linked to ischemic and epileptic damage in the brain. In addition, our evidence suggests that these channels are very important in skeletal development and skin differentiation. In the present study we will determine what regulates the function of the channels allowing them to release or uptake important signalling molecules. Using newly generated transgenic mouse models that lack one or two members of the pannexin family we will assess the skin and skeleton for abnormalities. Finally, since preliminary data suggests pannexin channels may be important in skeletal diseases, we will determine if the loss of one or more pannexins will lead to the acceleration or reversion of osteoarthritis.

It is a general requirement for normal function that adjacent cells within human tissues communicate directly with each other through special channels called gap junctions. In over 10 distinct human diseases these channels are either not produced by the cells (e.g. many cancers), or the proteins (connexins) that make up these channels contain mutations that inhibit their normal function (e.g. skin diseases, deafness, neuropathies). There are 21 connexin genes in the human genome and multiple connexins can be expressed and intermix in the same cells, leading to a complex array of gap junction channel types within tissues and organs. This is most evident in the epidermis of the skin where up to 10 different connexins are expressed and mutations in anyone of 5 of these connexins result in a variety of human skin diseases. Importantly, mutations in some of these same connexins cause additional disease burden which includes hearing loss and the disease symptoms tend to become evident during aging. In this study we will investigate how connexin mutations cause human diseases of the skin and why these disease manifest as a patient ages. Once it is better understood how connexin mutations cause diseases of the skin in aged patients it is anticipated that these findings could be translated to pre-clinical studies and possible treatments of gap junction-linked diseases.

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:

SEE E-mail

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	Material cultured at one time? (in Litres)	Containment Level
<i>DHS a Ecoli</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	<500 ml	Invitrogen <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>JM109</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	<500 ml	Invitrogen <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>E. coli</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		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Adenovirus</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		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Retrovirus</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		<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: Those competent cells are used for tranformation to amplify plasmid DNAs

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	skin biopsies	Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	genetically modified mice, fibroblasts	2006-101
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	HeLa, HEK293T, HEK, see append,	2	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	MDCK, N2A, BICR-M1Rk, see append,	2	Clonetic, ATCC
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No			

**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: These cell lines have previously been approved and records are on file with the Safety Office. See the appendix for a list of other cell lines in our laboratory.

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	ODDD patients and relatives	<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		<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	ODDD patients	<input type="checkbox"/> Yes		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2

Tissues (unpreserved)	and relatives	<input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)	ODDD patients and relatives	Not Applicable		Not Applicable

Additional Comments: Plans are to expand and seek other patients with connexin-linked diseases

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?
JM109	T-East, pcDNA3 (+), (-), pEGFP See appendix	Promega, Invitrogen, Clontech	connexin, pannexins	No	No	No

or equivalent if available.

and for the following strains of E. coli:
http://www.cdc.gov/od/ohs/CFIA_Ecoli_list.pdf

E. coli

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
Retrovirus Adenovirus	AP-2 pRNA-H1.1	Dr. J. Galipeau, McGill University GenScript	connexins, pannexins and N-cadherin	improved cell to environment and cell-cell communication

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

5.1 Is any work being conducted with prions or prion sequences?

NO YES

Additional Comments: _____

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 **Mice**

7.3 AUS protocol # **2006-101**

7.4 List the location(s) for the animal experimentation and housing. **West Valley and Dental Science Buildings**

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) **Cholera toxin**
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 **250ug/kg**

9.4 How much of the toxin or hormone is handled at one time*? **100ng/ml into culture medium**

9.5 How much of the toxin or hormone is stored*? **1mg**

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: **Cholera toxin will be used in MCF10A cell culture medium to facilitate the optimum growth conditions.**

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, cc **Cholera toxin from Cedarlane**
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 # **C-12-1146** 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 Dale W Laird **Date:** March 7, 2012

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: **May 19, 2011**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0017**

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:
The infectious material will be washed away if possible, the injured worker will be sent to emergency for assessment, an accident report will be filed and the biosafety office will be contacted to ensure that all necessary accident procedures are executed.

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 Dale W Laird **Date:** March 7, 2012

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:



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,

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

Subject: Re: Biological Agents Registry Form: Laird
From: Cindy Shao <Cindy.Shao@schulich.uwo.ca>
Date: 3/14/2012 9:57 AM
To: Jennifer Stanley <jstanle2@uwo.ca>

You are right, it is DH5 alpha. It is competent cells. Cindy

|| Jennifer Stanley <jstanle2@uwo.ca> 3/13/2012 5:12 PM >>>

Hi there -

I got your form in the mail - thanks.

Just a quick question - do you use DH5 alpha (on the form it says DHS but I imagine that this is a typo)?

Regards
Jennifer

E-mail

Info on cell Line(s)

Laird Biohazard registry Appendix:

Additional grant applications that have been submitted and are pending.

- 1) The role of Cx26 and Cx30 in aging skin (CIHR)
- 2) The cellular life and function of pannexins (CIHR)

Appendix 2.3, Types of established cell lines that are used in our laboratory:

Cell Type and specific cell lines

1. Human: CRL-7762, CRL-7804, CRL-7761, CCD-1074, SC.ZR75, Hs578T, MDA-MB-231, MDA-MB-435S, MCF10A, SK-HEP-1, HBL-100, MCF7, HaCaT, SUM159, SUM149, HeLa, HEK293, 293T
2. Rodent: C2C12, BL6, F0 melanomas, F10 melanomas, L10BIOBR-GFP, NRK, MC3T3-E1, NRK-52E, bEPD0670_4_B09, PC12, N2A, rat epidermal keratinocytes (REK), MDCK, BICR-M1Rk mammary tumor
3. Others: MDCK

All cell lines were obtained from ATCC or colleague laboratories and are handled under level 2 containment although many of these cell lines are designated containment level 1.

Appendix 4.0 Genetically-modified organisms and cell lines:

4.2: Plasmids:

pcDNA-mRFP from Addgene,
pTagRFP vector from Evrogen,
CMV-R-GECO1 from Addgene,
pRc/CMC from Invitrogen,

Tet-ON/off Cherry-vectors set from Clontech.

pEBTet GFP-vector from Germany, Mitochondria-RFP vector from Yale.

pEGFP fusion variants of connexins and pannexins and site-directed or truncated mutants of these genes.

Section 4.0

Cell Biology

ATCC® Number:

CRL-7762™

[Order this Item](#)

Price:

\$459.17 (non-profit list price)**[Log In](#) with customer # to see your price**[See New Benefits of ATCC Culture](#)

Designations: **TE 354.T**
Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Homo sapiens*

fibroblast

Morphology:



Source:

Organ: skin**Disease:** basal cell carcinoma

Permits/Forms:

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Amelogenin: X
 CSF1PO: 10,14
 D13S317: 11,14
 D16S539: 12

DNA Profile (STR):

D5S818: 10,13
 D7S820: 10
 TH01: 6
 TPOX: 8,12
 vWA: 14,17

Gender:

female

Comments:

The patient was sister of donor for ATCC CRL-7714.
 Part of the NBL Collection. Unlike other cell lines in the NBL Collection, this item has been fully accessioned by ATCC and is covered by the standard warranty.

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**Protocol:** Remove medium and rinse with 0.25% trypsin, 0.53 mM**Related Links ▶**[NCBI Entrez Search](#)[Cell Micrograph](#)[Make a Deposit](#)[Frequently Asked Questions](#)[Material Transfer Agreement](#) New![Technical Support](#)[Related Cell Culture Products](#)**[BioProducts](#)**

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Cell Biology

ATCC® Number:

CRL-7804™

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Price:

**Contact Customer Service
for pricing and availability**[See New Benefits of ATCC Culture](#)

Designations:

Hs 456.Sk

[Biosafety Level:](#)

1

Shipped:

flask

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

Morphology:

Source:

Organ: skin**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.

Cytogenetic Analysis:

modal number = 46; range = 44 to 47

Age:

80 years

Gender:

female

Ethnicity:

Caucasian

Comments:

The line was established from apparently normal tissue from a patient who had basal cell carcinoma (see ATCC CRL-7806). 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.](#)

Note: This item is distributed only within the 50 United States. It is not available for international distribution.

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:

Subcultivation Ratio: A subcultivation ratio of 1:2 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

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Cell Biology

ATCC® Number:

CRL-7761™

[Order this Item](#)

Price:

\$459.17 (non-profit list price)**[Log In](#) with customer # to see your price**[See New Benefits of ATCC Culture](#)

Designations:

TE 353.Sk

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

Morphology:

fibroblast

Source:

Organ: skin**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.

Gender:

female

Comments:

Patient was sister of donor for ATCC CRL-7714. 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**Related Links ▶**[NCBI Entrez Search](#)[Make a Deposit](#)[Frequently Asked Questions](#)[Material Transfer Agreement](#) New![Technical Support](#)[Related Cell Culture Products](#)**[BioProducts](#)**

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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

Cell Biology

ATCC® Number:	CRL-2090™	Order this Item	Price:	<p>\$551.00 (for-profit list price) \$459.17 (non-profit list price) Log In with customer # to see your price</p>
See New Benefits of ATCC Culture				
Designations:	CCD-1074Sk			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Homo sapiens</i>			
Morphology:	fibroblast			
Source:	<p>Organ: skin Disease: normal Cell Type: fibroblast</p>			
Permits/Forms:	<p>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.</p>			
Age:	42 years			
Gender:	female			
Comments:	<p>The line was established from skin taken from normal breast tissue removed at mastectomy. Cells senesce after approximately 26 population doublings.</p>			
Propagation:	<p>ATCC complete growth medium: Iscove's modified Dulbecco's medium, 90%; fetal bovine serum, 10%</p> <p>Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended</p>			
Subculturing:	<p>Medium Renewal: Every 3 to 4 days Remove spent medium, add fresh 0.25% trypsin, 0.03% EDTA solution, rinse and remove trypsin. Let the culture sit at room temperature (or at 37C) for 2 to 5 minutes. Add fresh medium, aspirate and dispense into new flasks. Subculture every 6 to 8 days.</p>			
Preservation:	Culture medium, 95%; DMSO, 5%			

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Cell Biology

ATCC® Number:

CRL-1500™

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Price:

\$431.00 (for-profit list price)
 \$359.17 (non-profit list price)

[Log In](#) with customer # to see your price

[See New Benefits of ATCC Culture](#)

Designations:

ZR-75-1

Depositors:

LW Engel

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

epithelial

Morphology:

**Organ:** mammary gland; breast**Tissue:** duct

Source:

Disease: ductal carcinoma**Derived from metastatic site:** ascites**Cell Type:** epithelial

Cellular Products:

mucin (apomucin, MUC-1, MUC-2)

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC

Permits/Forms:

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.

Receptors:

estrogen receptor, expressed

Tumorigenic:

Yes

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DNA Profile (STR): Amelogenin: X
 CSF1PO: 10,11
 D13S317: 9
 D16S539: 11
 D5S818: 13
 D7S820: 10,11
 THO1: 7,9.3
 TPOX: 8

Cell Biology

ATCC® Number:

HTB-126™

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Price:

\$431.00 (for-profit list price)
\$359.17 (non-profit list price)

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[See New Benefits of ATCC Culture](#)

Designations:

Hs 578T

Depositors:

AJ Hackett

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens
epithelial

Morphology:



Source:

Organ: mammary gland; breast**Disease:** carcinoma

Permits/Forms:

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Receptors:

estrogen receptor, not expressed [[1119](#)]

Tumorigenic:

No

Amelogenin: X

CSF1PO: 13

D13S317: 11

D16S539: 9,12

DNA Profile (STR):

D5S818: 11

D7S820: 10

THO1: 9,9.3

TPOX: 8

vWA: 17

Cytogenetic Analysis:

Number of cells examined = 50; Modal Chromosome Number = 59 with a range of 50 to 77; Polyploidy Rate = 33.8%
Composite karyotype: 50-77 <3n> X, -1, del(1)(q12), -2, del(2)(? q36), der(3)t(3;15)(q10;p10), -4, -5, der(5)t(5;8)(p10;q10), -6, i(6)(p10), +8, -9, -10, -11, del(11)(p12), -12, -13, -14, -15, -15, -16, -17, -17, -17, i(17)(q10), -18, -19, der(19)(19pter<-q13::5q13<-qter), +22, +3 mar[cp12]

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Cell Biology

ATCC® Number: **HTB-26™** [Order this Item](#) Price:

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Designations: **MDA-MB-231**

Depositors: R Cailleau

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens*

epithelial

Morphology:



Organ: mammary gland; breast

Disease: adenocarcinoma

Source:

Derived from metastatic site: pleural effusion

Cell Type: epithelial

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.

Permits/Forms:

Applications: transfection host

Receptors: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Yes

Amelogenin: X

CSF1PO: 12,13

D13S317: 13

D16S539: 12

DNA Profile (STR): D5S818: 12

D7S820: 8,9

THO1: 7,9.3

TPOX: 8,9

vWA: 15,18

Cytogenetic Analysis:

The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

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Cell Biology

ATCC® Number:

HTB-129™

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Designations:

MDA-MB-435S

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

spindle shaped

Morphology:

**Organ:** previously described as: mammary gland; breast

Source:

Disease: previously described as ductal carcinoma**Derived from metastatic site:** pleural effusion

Cellular Products:

tubulin; actin

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.

Permits/Forms:

Isolation:

Isolation date: 1976

Tumorigenic:

No

Amelogenin: X

CSF1PO: 11

D13S317: 12

D16S539: 13

DNA Profile (STR):

D5S818: 12

D7S820: 8,10

THO1: 6,7

TPOX: 8,11

vWA: 16,18

modal number = 56; range = 55 to 62

Cytogenetic Analysis:

The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal

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Cell Biology

ATCC® Number:

CRL-10317™

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Price:

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Designations: **MCF 10A**
 Depositors: Michigan Cancer Foundation
Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Homo sapiens*
 Morphology: epithelial

Source: **Organ:** mammary gland; breast
Disease: fibrocystic disease
Cell Type: epithelial

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:** August 22, 1984

Applications: transfection host

Tumorigenic: No

Amelogenin: X
 CSF1PO: 10,12
 D13S317: 8,9
 D16S539: 11,12

DNA Profile (STR): D5S818: 10,13
 D7S820: 10,11
 THO1: 8,9.3
 TPOX: 9,11
 vWA: 15,17

Isoenzymes: AK-1, 1 [[23084](#)]
 ES-D, 1 [[23084](#)]
 G6PD, B [[23084](#)]
 GLO-I, 1-2 [[23084](#)]
 PGM1, 1-2 [[23084](#)]
 PGM3, 1 [[23084](#)]

Age: 36 years

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Cell Biology

ATCC® Number:

HTB-52™

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Price:

\$359.17 (non-profit list price)

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Designations:

SK-HEP-1

Depositors:

G Trempe, LJ Old

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Homo sapiens

Morphology:

epithelial

Source:

Organ: liver**Tissue:** ascites**Disease:** adenocarcinoma

Permits/Forms:

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Restrictions:

The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation:

Isolation date: 1971

Applications:

transfection host ([Roche Transfection Reagents](#))

Tumorigenic:

Yes

DNA Profile (STR):

Amelogenin: X
 CSF1PO: 11,12
 D13S317: 8,12
 D16S539: 12
 D5S818: 10,13
 D7S820: 8,11
 THO1: 7,9
 TPOX: 9

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Cell Biology

ATCC® Number: **HTB-22™** [Order this Item](#) Price:

\$359.17 (non-profit list price)

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Designations: **MCF7**
 Depositors: CM McGrath
Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Homo sapiens*

epithelial

Morphology:



Organ: mammary gland; breast

Disease: adenocarcinoma

Source:

Derived from metastatic site: pleural effusion

Cell Type: epithelial

Cellular Products:

insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC

Permits/Forms:

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:

estrogen receptor, expressed

Antigen Expression:

Blood Type O; Rh+

DNA Profile (STR): Amelogenin: X

CSF1PO: 10

D13S317: 11

D16S539: 11,12

D5S818: 11,12

D7S820: 8,9

THO1: 6

TPOX: 9,12

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Cell Biology

ATCC® Number:

CCL-2™

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Price:

\$359.17 (non-profit list price)**[Log In](#) with customer # to see your price**[See New Benefits of ATCC Culture](#)

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

epithelial

Morphology:

**Organ:** cervix

Source:

Disease: adenocarcinoma**Cell Type:** epithelial

Permits/Forms:

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Applications:

transfection host
 screening for Escherichia coli strains with invasive potential
 Human adenovirus 3
 Encephalomyocarditis virus

Virus Susceptibility:

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

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Cell Biology

ATCC® Number:

CRL-1573™

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Price:

\$359.17 (non-profit list price)**[Log In](#) with customer # to see your price**[See New Benefits of ATCC Culture](#)

Designations: **293 [HEK-293]**

Depositors: FL Graham

Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens*
epithelial

Morphology:



Source:

Organ: embryonic kidney

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.

Permits/Forms:

Restrictions:

These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications:

efficacy testing
transfection host
viruscide testing

Receptors:

vitronectin, expressed

Tumorigenic:

YES

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

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Cell Biology

ATCC® Number:

CRL-11268™

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Price:

\$359.17 (non-profit list price)

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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* deposited as human
 Morphology: epithelial

Source:

Organ: kidney

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.

Permits/Forms:

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 Rockefeller University, Office of Technology Transfer, 1230 York Avenue, New York, NY 10065 Attn: Kathleen A. Denis, Associate Vice President Technology Transfer.

Applications:

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.

Antigen Expression:

SV40 T antigen [\[45408\]](#)

Amelogenin: X

CSF1PO: 11, 12

D13S317: 12, 14

D16S539: 9, 13

DNA Profile (STR):

D5S818: 8, 9

D7S820: 11

TH01: 7, 9, 2

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Cell Biology

ATCC® Number:	CRL-1772™	Order this Item	Price:	<p>\$359.17 (non-profit list price) Log In with customer # to see your price</p> <p style="border: 1px solid black; padding: 2px; text-align: center;">See New Benefits of ATCC Culture</p> <p>Related Links ▶ NCBI Entrez Search Cell Micrograph Make a Deposit Frequently Asked Questions Material Transfer Agreement New! Technical Support Related Cell Culture Products</p> <p> Product Information Sheet</p> <p>BioProducts Cell, microbial and molecular genomics products for the life sciences</p> <p>BioServices Bio-materials management; basic repository to complex partnership-level services</p> <p>BioStandards Biological Reference Material and Consensus Standards for the life science community</p>
Designations:	C2C12			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Mus musculus</i> myoblast			
Morphology:				
Source:	<p>Strain: C3H Tissue: muscle Cell Type: myoblast;</p> <p>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.</p>			
Permits/Forms:				
Applications:	<p>transfection host</p> <p>This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. Tested and found negative for ectromelia virus (mousepox).</p>			
Comments:	<p>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</p>			
Propagation:	<p>Protocol: IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT. Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture. Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.</p> <ol style="list-style-type: none"> 1. Remove and discard culture medium. 			

Cell Biology

ATCC® Number:	CRL-6322™	Order this Item	Price:	\$359.17 (non-profit list price) Log In with customer # to see your price
				See New Benefits of ATCC Culture
Designations:	B16-F0			Related Links ▶
Biosafety Level:	1			NCBI Entrez Search
Shipped:	frozen			Make a Deposit
Medium & Serum:	See Propagation			Frequently Asked Questions
Growth Properties:	adherent			Material Transfer Agreement New!
Organism:	<i>Mus musculus</i>			Technical Support
Morphology:	mixture of spindle-shaped and epithelial-like cells			Related Cell Culture Products
Source:	Organ: skin Strain: C57BL/6J Disease: melanoma			BioProducts
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.			Cell, microbial and molecular genomics products for the life sciences
Applications:	transfection host (Roche Transfection Reagents)			BioServices
Tumorigenic:	Yes			Bio-materials management; basic repository to complex partnership-level services
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 (CO ₂), 5% Temperature: 37.0°C			BioStandards
				Biological Reference Material and Consensus Standards for the life science community

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
- Subculturing:

Cell Biology

ATCC® Number:

CRL-6475™

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Price:

\$359.17 (non-profit list price)**[Log In](#) with customer # to see your price**[See New Benefits of ATCC Culture](#)

Designations:

B16-F10

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Mus musculus

mixture of spindle-shaped and epithelial-like cells

Morphology:

**Organ:** skin

Source:

Strain: C57BL/6J**Disease:** melanoma

Permits/Forms:

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Applications:

transfection host

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Temperature: 37.0°C**Atmosphere:** air, 95%; carbon dioxide (CO₂), 5%**Related Links ▶**[NCBI Entrez Search](#)[Cell Micrograph](#)[Make a Deposit](#)[Frequently Asked Questions](#)[Material Transfer Agreement](#) New![Technical Support](#)[Related Cell Culture Products](#)[Product Information Sheet](#)**BioProducts**

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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

Subculturing:



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L10BIOBR-GFP from American Type Culture Collection (ATCC)



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L10BIOBR-GFP from American Type Culture Collection (ATCC)



Product	L10BIOBR-GFP
Company	American Type Culture Collection (ATCC)
Price	REQUEST INFO Request Information for this Product Pricing Info
More Information	View Company Product Page
Catalog Number	CRL-2770
Quantity	1 vial

Description

The L10BIOBR-GFP cell line was derived by infecting the immortalized murine melanocyte cell line L10BIOBR with pDIVA-GFP. The vector contains the puromycin resistant gene. The cells were selected on medium containing puromycin. Both cell lines, L10BIOBR-GFP (CRL-2770?) and L10BIOBR-MAPKK (ATCC CRL-2771?) will serve as controls for oncogenic transformation and signal transduction studies for melanoma [PubMed: 12514183].

Cell Biology

ATCC® Number:

CRL-6509™

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Price:

\$459.17 (non-profit list price)

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Designations:

NRK

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Rattus norvegicus

Morphology:

epithelial

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Source:

Organ: kidney**Strain:** Osborne-Mendel**Disease:** normal

Permits/Forms:

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Applications:

transfection host ([Roche Transfection Reagents](#))

Cytogenetic Analysis:

modal number = 44; range = 39 to 44

Age:

adult

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**[BioProducts](#)**

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Subculturing:

Protocol: Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:12 is recommended

Medium Renewal: Every 2 to 3 days

Cell Biology

ATCC® Number:

CRL-2593™

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Price:

\$431.00 (for-profit list price)

\$359.17 (non-profit list price)

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Designations: MC3T3-E1 Subclone 4

Depositors: RT Franceschi

[Biosafety Level:](#) 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Mus musculus*

Morphology: fibroblast

Organ: bone**Strain:** C57BL/6**Tissue:** calvaria**Cell Type:** preosteoblast;

Source:

Cellular Products: collagen [[51540](#)]

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Permits/Forms:

These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behavior similar to primary calvarial osteoblasts.

Applications:

The MC3T3-E1 Subclone 4 (ATCC [CRL-2593](#)) and the MC3T3 Subclone 14 (ATCC [CRL-2594](#)) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate.

Tumorigenic:

Yes

Age:

newborn

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A series of subclones were isolated from the cloned but phenotypically heterogeneous MC3T3-E1 cell line. The subclones were selected for high or low osteoblast differentiation and mineralization after growth in medium containing ascorbic acid. The MC3T3-E1 Subclone 4 (ATCC [CRL-2593](#)) and the MC3T3 Subclone 14 (ATCC [CRL-2594](#)) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate. They form a well mineralized extracellular

Cell Biology

ATCC® Number:

CRL-1571™

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Price:

\$431.00 (for-profit list price)
 \$359.17 (non-profit list price)
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Designations: NRK-52E
 Depositors: JE DeLarco
Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Rattus norvegicus* deposited as *Rattus* sp.
 Morphology: epithelial

Source: **Organ:** kidney
Disease: normal
 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.

Permits/Forms:

Applications: transfection host
 Receptors: epidermal growth factor (EGF); multiplication stimulating activity (MSA)

Propagation: **ATCC complete growth medium:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 95%; bovine calf serum, 5%
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

Subculturing: **Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.02% 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. Centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh culture medium, aspirate and dispense into new culture vessels.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended

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Cell Biology

ATCC® Number:

CRL-1721™

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Price:

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\$359.17 (non-profit list

price)

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Designations:

PC-12

Depositors:

B Patterson

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

floating clusters; few scattered lightly attached cells.

Organism:

Rattus norvegicus deposited as *Rattus* sp.

small irregularly shaped cells

Morphology:



Source:

Organ: adrenal gland**Disease:** pheochromocytoma

Cellular Products:

catecholamines; dopamine; norepinephrine

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Permits/Forms:

Applications:

transfection host

Receptors:

nerve growth factor (NGF), expressed

Tumorigenic:

Yes

Cytogenetic Analysis:

40 chromosomes; 38 autosomes plus XY [\[1163\]](#)

Gender:

male

The PC-12 cell line was derived from a transplantable rat pheochromocytoma.

Comments:

The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. The cells do not synthesize epinephrine.

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:

Propagation:

- heat-inactivated horse serum to a final concentration of 10%
- fetal bovine serum to a final concentration of 5%

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Cell Biology

ATCC® Number:

CCL-131™

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Price:

\$431.00 (for-profit list price)

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Designations:

Neuro-2a

Depositors:

RJ Klebe

[Biosafety Level:](#)

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Mus musculus

neuronal and amoeboid stem cells

Morphology:

**Organ:** brain**Strain:** A

Source:

Disease: neuroblastoma**Cell Type:** neuroblast;

Cellular Products:

acetylcholinesterase

tubulin

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Permits/Forms:

Applications:

transfection host

Herpes simplex virus

Virus Susceptibility:

Vesicular stomatitis virus

Human poliovirus 1

Antigen Expression:

H-2, a haplotype; *Mus musculus*, expressed

modal number = 95; range = 59 to 193.

Cytogenetic Analysis:

Karyotype unstable within a stemline range of 94 to 98 chromosomes. All the cells contain 6 to 10 large chromosomes with median or submedian centromeres and 2 to 4 minute chromosomes.

GenoType:

albino

Clone Neuro-2a was established by R.J. Klebe and F.H. Ruddle from a spontaneous tumor of a strain A albino mouse. This tumor line, designated C1300, was obtained from the Jackson Laboratory, Bar Harbor, Maine [22161]. Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells. The cell line has been used for studies on the mechanism of vinblastine precipitation of microtubular protein. the kinetics of

Comments:

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Cell Biology

ATCC® Number:

CCL-34™

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Price:

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Designations:

MDCK (NBL-2)

Depositors:

S Madin, NB Darby

Biosafety Level:

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Canis familiaris

epithelial

Morphology:



Source:

Organ: kidney**Disease:** normal

Cellular Products:

keratin

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Permits/Forms:

Isolation:

Isolation date: September, 1958

Applications:

transfection host

Human Coxsackievirus B 5

Reovirus type 2

Adeno-associated virus 4

Vaccinia virus

Virus Susceptibility:

Vesicular stomatitis virus

Adeno-associated virus 5

Human Coxsackievirus B 3

Human Coxsackievirus B 4

Human poliovirus 2

Cytogenetic Analysis: Polyploidy 0.2%. Two large submetacentric chromosomes noted, presumably X chromosomes, and one or two additional chromosomes with median or submedian centromeres.

Age:

adult

Gender:

female

Comments:

The MDCK cell line was derived from a kidney of an apparently normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby. The cells are positive for keratin by immunoperoxidase staining. MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its

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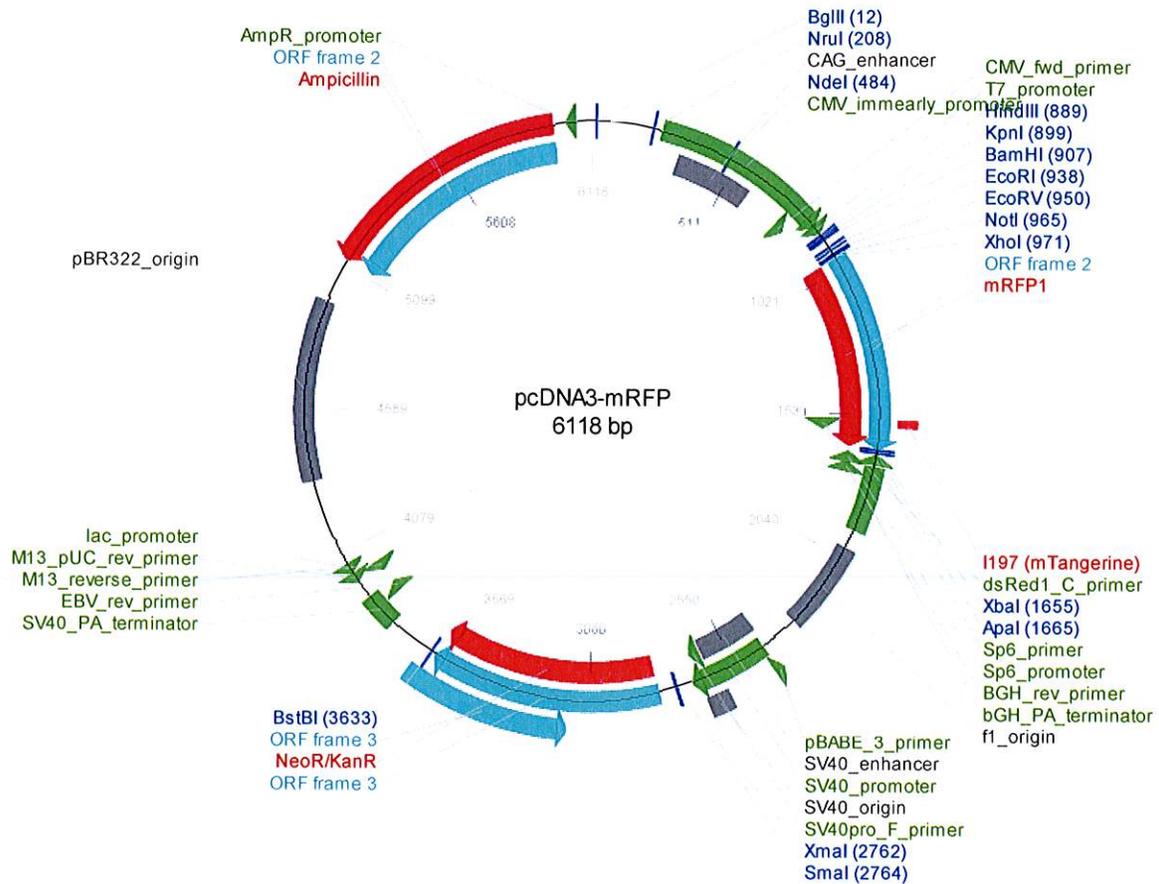
[Browse](#) > [Doug Golenbock](#) > [Fluorescent Protein Plasmids](#) > pcDNA3-mRFP

Plasmid 13032: pcDNA3-mRFP

Gene/insert name: Monomer Red Fluorescent Protein
Alt name: mRFP
Insert size: 678
GenBank ID: AF506027
Vector backbone: pcDNA3
([Search Vector Database](#))
Backbone manufacturer: Invitrogen
Vector type: Mammalian Expression
Backbone size w/o insert: 5446
Cloning site 5': XhoI
Site destroyed during cloning: No
Cloning site 3': XbaI
Site destroyed during cloning: No
5' sequencing primer: T7 [List of Sequencing Primers](#)
3' sequencing primer: GTCTTGAGTTGCCGTCGTC
Bacterial resistance: Ampicillin
Growth strain: DH5alpha
Growth temperature (°C): 37
High or low copy: High Copy
Selectable markers: Neomycin
Person or lab that originally cloned the gene/insert: mRFP is from Roger Tsien, UCSD.
Sequence: [View sequences \(2\)](#)
Principal Investigator: Doug Golenbock
Terms and Licenses: [MTA](#)
[Clontech Limited Use Label License](#)

Comments: mRFP cloned into the old, discontinued pcDNA3 vector from Invitrogen.

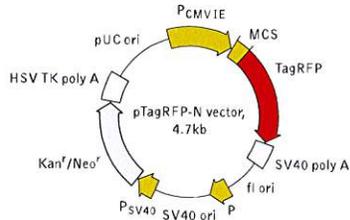
Addgene has sequenced a portion of this plasmid for verification. Click [here](#) for the sequencing result.



Feature Name	Start	End
CMV_immearily_promoter	236	852
CAG_enhancer	315	602
CMV_fwd_primer	769	789
T7_promoter	863	881
mRFP1	980	1651
I197 (mTangerine)	1553	1579
dsRed1_C_primer	1569	1592
Sp6_primer	1688	1671
Sp6_promoter	1689	1672
BGH_rev_primer	1725	1708
bGH_PA_terminator	1711	1938
f1_origin	2001	2307
pBABE_3_primer	2441	2421
SV40_enhancer	2643	2427
SV40_promoter	2439	2708
SV40_origin	2607	2684
SV40pro_F_primer	2669	2688
NeoR/KanR	2826	3614
SV40_PA_terminator	3794	3913
EBV_rev_primer	3882	3901
M13_reverse_primer	3975	3957
M13_pUC_rev_primer	3996	3974
lac_promoter	4039	4010
pBR322_origin	4967	4348
Ampicillin	5982	5122
AmpR_promoter	6052	6024

pTagRFP-N vector

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



For vector sequence, please visit our Web site at <http://www.evrogen.com/support/vector-info.shtml>

Multiple cloning site (MCS)

$\xrightarrow{\text{MCS}}$

 ... G. CTA. CCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. G ...

 $\xrightarrow{\text{TagRFP}}$

* - not unique site.

Location of features

P_{CMV IE}: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-671
TagRFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; **Stop codon:** 1390-1392
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1542-1547 & 1571-1576
mRNA 3' ends: 1580 & 1592
f1 single-strand DNA origin: 1639-2094
Eukaryotic promoter for expression of Kan['] gene
-35 region: 2156-2161; **-10 region:** 2179-2184
Transcription start point: 2191
SV40 origin of replication: 2435-2570
SV40 early promoter
Enhancer (72-bp tandem repeats): 2268-2339 & 2340-2411
21-bp repeats: 2415-2435, 2436-2456 & 2458-2478
Early promoter element: 2491-2497
Major transcription start points: 2487, 2525, 2531 & 2536
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2619-2621; **Stop codon:** 3411-3413
G→A mutation to remove Pst I site: 2801
C→A (Arg to Ser) mutation to remove BssH II site: 3147
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3649-3654 & 3662-3667
pUC plasmid replication origin: 3998-4641

References

Gorman (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*, Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143-190.
 Haas et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
 Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res.* 15 (20): 8125-8148 / pmid: 3313277

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Product	Cat.#	Size
pTagRFP-N vector	FP142	20 µg
The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	TagRFP	
Reporter codon usage	mammalian	
Promoter for TagRFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	TagRFP expression in mammalian cells; generation of fusions to the TagRFP N-terminus	

Vector description

pTagRFP-N is a mammalian expression vector encoding red (orange) fluorescent protein TagRFP. The vector allows generation of fusions to the TagRFP N-terminus and expression of TagRFP fusions or TagRFP alone in eukaryotic (mammalian) cells.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagRFP sequence [Kozak 1987]. Multiple cloning site (MCS) is located between P_{CMV IE} and TagRFP coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P_{CMV IE}) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo[']) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan[']) in *E. coli*. Kan[']/Neo['] gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Generation of TagRFP-tagged fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the TagRFP N-terminus when inserted in the same reading frame as TagRFP and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TagRFP, when transfected into eukaryotic (mammalian) cells.

Note: The plasmid DNA was isolated from dam⁻-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam⁺ host and make fresh DNA.

Expression in mammalian cells

pTagRFP-N vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 [Gorman 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.



A better way to share plasmids

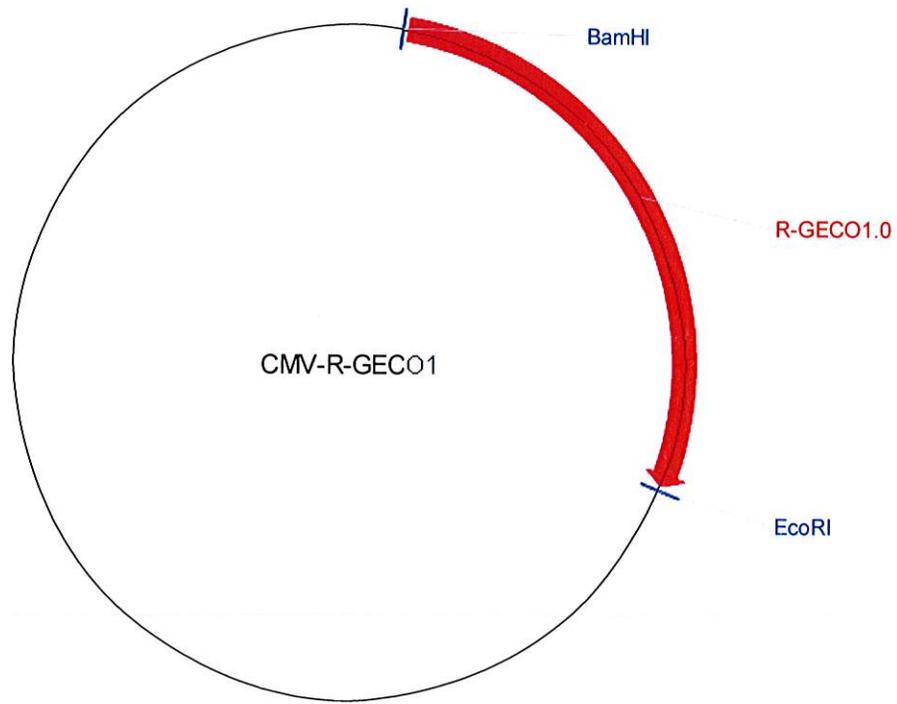
[Browse](#) > [Robert Campbell](#) > [Zhao et al](#) > CMV-R-GECO1

Plasmid 32444: **CMV-R-GECO1**

Gene/insert name: R-GECO1.0
Alt name: red intensimetric genetically encoded Ca²⁺-indicators for optical imaging
Insert size: 1254
Species: synthetic construct
GenBank ID: JN258411
Mutation: Substitutions relative to the mApple-derived analogue of GCaMP3:
T47A/L60P/E61V/S63V/E64S/R81G/K83R/Y134C/M158L/N164aD/V228A/
S290P/I366F/K380N/S404G/N414D/E430V
Vector backbone: Customized Vector
([Search Vector Database](#))
Vector type: Mammalian Expression
Backbone size w/o insert: 3200
Promoter: CMV
Cloning site 5': BamHI
Site destroyed during cloning: No
Cloning site 3': EcoRI
Site destroyed during cloning: No
5' sequencing primer: TAATACGACTCACTATAGGG [List of Sequencing Primers](#)
3' sequencing primer: TAGAAGGCACAGTCGAGG
Bacterial resistance: Ampicillin
Growth strain: DH10B
Growth temperature (°C): 37
High or low copy: High Copy
Sequence: [View sequences \(3\)](#)
Principal Investigator: Robert Campbell
Terms and Licenses: [MTA](#)

Comments: Note: Could not make stable cell line using this vector.

Addgene has sequenced a portion of this plasmid for verification. Click [here](#) for the sequencing result.



Article: [An Expanded Palette of Genetically Encoded Ca²⁺ Indicators](#). Zhao et al (Science. 2011 Sep 8. [PubMed](#))

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 32444" in your Materials and Methods section.

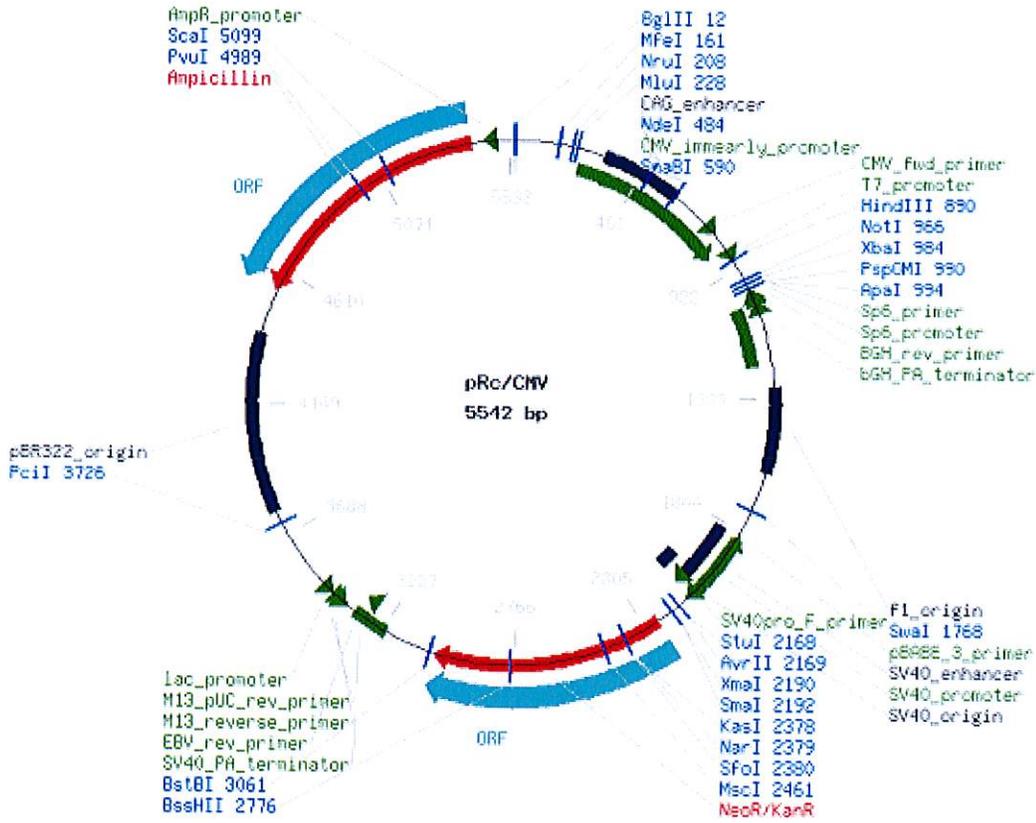


Community

 **Vector Database** > pRc/CMV **addgene** **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by [Addgene](#), and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pRc/CMV
Alt Names	RcCMV
Source/Vendor	Invitrogen
Plasmid Type	Mammalian
Promoter	CMV
Plasmid Size	5542
Sequencing Primer	T7/Sp6
Bacterial Resistance	Amp
Mammalian Selection	Neomycin
Catalog Number	V75020
Plasmid Sequence	View Sequence





Inducible Systems

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- ▶ Inducible Protein Stabilization
- ▶ Tetracycline-Inducible Expression
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 - [Tet-Inducible shRNA](#)
 - [TetR Monoclonal Antibody](#)
 - ▶ Viral Systems

Tet-On 3G Tetracycline-Inducible Expression Systems

The Tet-On 3G Tetracycline-Inducible Gene Expression Systems are the 3rd generation of the most powerful, versatile, and widely cited inducible mammalian expression systems available. The Tet-On 3G system offers a significant improvement over Tet-On and Tet-On Advanced with significantly **reduced basal expression** and **increased sensitivity to doxycycline**, a tetracycline analogue.

For more details, download our [Tet-On 3G technical note](#).

Lowest Background Ever

Mutations within the P_{TRE3G} promoter have [reduced background expression](#) from this tetracycline inducible promoter by 5–20-fold compared to our previous tightest promoter, P_{Tight} .

Highest Sensitivity

Mutations to create the Tet-On 3G transactivator have [significantly increased its sensitivity](#) to the inducer doxycycline (Dox).

Highest Fold Induction

Fold induction refers to the difference between the induced and uninduced states. Since Tet-On 3G retains the high maximal expression demonstrated by all Tet-On and Tet-Off systems but has significantly reduced basal expression compared to its predecessors, the fold induction levels are far higher.

Choice of Tet-On 3G Vector Formats

There are a range of [available Tet-On 3G vector formats](#):

- The Tet-On 3G Inducible Expression System (EF1 α Version), our EF-1 alpha promoter version of the Tet-On 3G system, provides for consistent long-term expression of the Tet-On 3G transactivator, even in cell types known for their tendency to silence a CMV promoter over time, such as hematopoietic cells and stem cells.
- Using IRES technology, we've married very tight gene expression control to a bright red fluorescent protein (mCherry) in the Tet-On 3G Inducible Expression System (with mCherry) and a bright green fluorescent protein (ZsGreen1) in the Tet-On 3G Inducible Expression System (with ZsGreen1). If your cells turn red or green after adding Dox, this confirms that your gene has been turned on and that you have selected a high-performing inducible clone.
- Retroviral and lentiviral formats include the P_{TRE3GV} promoter, which is optimized for use in retroviral and lentiviral vectors. They are supplied with complete viral packaging systems.

Use Tetracycline-Approved Serum for Optimal Results

With the greatly increased sensitivity of the Tet-On 3G System, it is more important than ever that the serum you use for your studies is guaranteed to be tetracycline-free. Only Clontech performs actual inducibility tests on a sensitive Tet inducible cell line in order to provide an absolute guarantee that your serum will not affect basal expression in your Tet-On 3G experiments. Each Tet-On 3G System is supplied with 50 ml of our premium Tet-Approved FBS.

Fast set-up of doxycycline-inducible protein expression in human cell lines with a single plasmid based on Epstein–Barr virus replication and the simple tetracycline repressor

Markus Bach¹, Silke Grigat¹, Barbara Pawlik¹, Christian Fork¹, Olaf Utermöhlen², Sonia Pal¹, David Banczyk¹, Andreas Lazar¹, Edgar Schömig^{1,3} and Dirk Gründemann^{1,3}

1 Department of Pharmacology, University of Cologne, Germany

2 Institute for Medical Microbiology, Immunology, and Hygiene, University of Cologne, Germany

3 Center for Molecular Medicine, University of Cologne (CMMC), Germany

Keywords

doxycycline; Epstein–Barr virus; polyadenylation; regulated protein expression; tetracycline repressor

Correspondence

D. Gründemann, Department of Pharmacology, University of Cologne, Gleueler Straße 24, 50931 Cologne, Germany

Fax: +49 221 478 5022

Tel: +49 221 478 7455

E-mail: dirk.gruendemann@uni-koeln.de

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We have developed a novel plasmid vector, **pEBTetD**, for full establishment of doxycycline-inducible protein expression by just a single transfection. pEBTetD contains an Epstein–Barr virus origin of replication for stable and efficient episomal propagation in human cell lines, a cassette for continuous expression of the simple tetracycline repressor, and a cytomegalovirus-type 2 tetracycline operator (tetO2)-tetO2 promoter. As there is no integration of vector into the genome, clonal isolation of transfected cells is not necessary. Cells are thus ready for use 1 week after transfection; this contrasts with 3–12 weeks for other systems. Adequate regulation of protein expression was accomplished by abrogation of mRNA polyadenylation. In northern analysis of seven cDNAs coding for transport proteins, pools of transfected human embryonic kidney 293 cells showed on/off mRNA ratios in the order of 100 : 1. Cell pools were also analyzed for regulation of protein function. With two transport proteins of the plasma membrane, the on/off activity ratios were 24 : 1 and 34 : 1, respectively. With enhanced green fluorescent protein, a 23 : 1 ratio was observed based on fluorescence intensity data from flow cytometry. The unique advantage of our system rests on the unmodified tetracycline repressor, which is less likely, by relocation upon binding of doxycycline, to cause cellular disturbances than chimera of tetracycline repressor and eukaryotic transactivation domains. Thus, in a comprehensive comparison of on- and off-states, a steady cellular background is provided. Finally, in contrast to a system based on Flp recombinase, the set-up of our system is inherently reliable.

The function of human proteins is commonly analyzed by heterologous expression in cultured cell lines. Regulated expression, i.e. a system to switch on expression on demand, has clear advantages over constitutive expression. With constitutive expression, cells may die during antibiotic selection because of toxic effects of

the expressed protein [1]. Also, for a close match of backgrounds, it is better to compare two states of a single cell line rather than two separately transfected and selected cell lines.

Several widely used systems for regulated expression in mammalian cell lines are based on the tetracycline

Abbreviations

CMV, cytomegalovirus; EBV, Epstein–Barr virus; ETTh, ergothioneine transporter from human; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; eGFP, enhanced green fluorescent protein; MPP⁺, 1-methyl-4-phenylpyridinium; rTA, reverse tetracycline-controlled transcriptional activator; tetO2, type 2 tetracycline operator; TetR, tetracycline repressor; tTA, tetracycline-controlled transcriptional activator; tTS, tetracycline-controlled transcriptional silencer.

repressor (TetR) [2,3]. Current systems require two or three rounds of transfection of separate plasmids and clonal isolation, which makes setting-up an inducible cell line a protracted (at least 3 weeks if one buys cell lines prepared for the final round, or 12 weeks if one starts from scratch) and expensive procedure. In order to avoid clonal selection in the final round of transfection, the Flp-InTM-T-RexTM system (Invitrogen, Karlsruhe, Germany) may be used. Here, in the first transfection, a Flp recombinase target site is introduced randomly into the genome; *tetR* follows in the second transfection. In the final transfection, Flp recombinase from a cotransfected plasmid is used to integrate the plasmid for protein expression into the target site. Since the open reading frame for hygromycin resistance on the expression plasmid lacks a start codon, random integration into the genome does not yield resistant cells. This leads to a uniform pool of transfected cells; clonal selection is unnecessary. Unfortunately, despite intensive scrutiny, we and others have experienced a high failure rate (~90% of all transfections) with this system, where no clones at all were generated in the end, even with the positive control plasmid supplied.

The major bottleneck in stable transfection of cells arises from the low frequency of stable plasmid integration into genomic DNA. At best, only ~0.001% of cells generate clones. Expansion of the few survivors takes weeks, particularly with selection and functional testing of individual clones. In contrast, plasmids with an Epstein-Barr virus (EBV) origin of replication *oriP* in the presence of EBV-encoded EBNA-1 protein are continuously propagated in ~1% of initially transfected cells [4]. EBV plasmid replication has been demonstrated for a large variety of human cell lines; primate and canine cell lines may also be used [5]. It has been extensively documented that the plasmids are maintained episomally (5–10 copies per cell, e.g. for 293 cells), i.e. they do not integrate into genomic DNA [5–7]. Hence, it is expected that clone-specific effects of the genetic neighbourhood (positional effects) on protein expression are avoided [8]. It thus becomes feasible to work with transfected cell pools instead of single cell clones. Altogether, it would save much time to employ an EBV vector that carries all elements necessary for doxycycline-regulated gene expression on a single plasmid.

We have recently developed a substrate search strategy for integral membrane transport proteins termed 'LC-MS difference shading' [9]. Our strategy is based on comparative analysis of lysates of cells both with and without transporter expression. The expression of all other proteins in the two cell populations should match as closely as possible. Thus, for us, a suitable

system for regulated gene expression must provide an identical background.

EBV-derived single-plasmid systems for tetracycline-regulated gene expression have been described previously; these are based on the TetR-VP16 fusion proteins tetracycline-controlled transcriptional activator (tTA) [10] or reverse tetracycline-controlled transcriptional activator (rtTA) [8]. A third system [11] is based on concomitant expression of two fusion proteins, i.e. rtTA2^S-M2, which contains three tandem repeat VP16 minimal activation domains, and tetracycline-controlled transcriptional silencer (tTS)^{KRAB}, which contains the N-terminus of the KRAB repressor domain of the mammalian Kox1 protein. However, it is well known that transactivator domains, such as VP16, interact with a variety of transcription factors [12,13]. Indeed, analysis of expression levels in stably transfected HeLa cells suggests that in high numbers, even TetR fusion proteins based on VP16 minimal activation domains are toxic [12]. Thus, relocation of TetR fusion proteins upon binding of inducer can be expected to cause secondary background differences. Pronounced alteration of rtTA expression levels after addition of inducer would promote further differences [8]. Another single-plasmid EBV system based on regulation by temperature shift (29 °C versus 37 °C) was also expected to display disturbing background differences [14]. Instead we opted to utilize continuous expression of the unmodified TetR. The original tetracycline repressor simply binds to a tandem of the type 2 tetracycline operator (*tetO2*) operator and thus blocks transcription from the upstream cytomegalovirus (CMV) promoter [15]. Addition of tetracycline or doxycycline to the culture medium turns on expression: the inducer binds to the repressor, which then dissociates from the operator. From the lack of interaction of the unmodified TetR with mammalian transcription factors, a steady background can be expected. In addition, evidence from yeast suggests that the inducer doxycycline itself has no significant effect on global transcription levels [16]. Thus, it was our aim to develop a single-plasmid EBV vector for doxycycline-regulated gene expression based on the simple TetR. Such a vector has not been reported before.

Results and Discussion

We have constructed plasmid pEBTet which links all elements necessary for doxycycline-inducible expression with the EBV origin of replication (Fig. 1). The orientation of elements in this vector appears to be critical, since an otherwise identical plasmid with the *tetR* cassette in reverse orientation yielded no viable

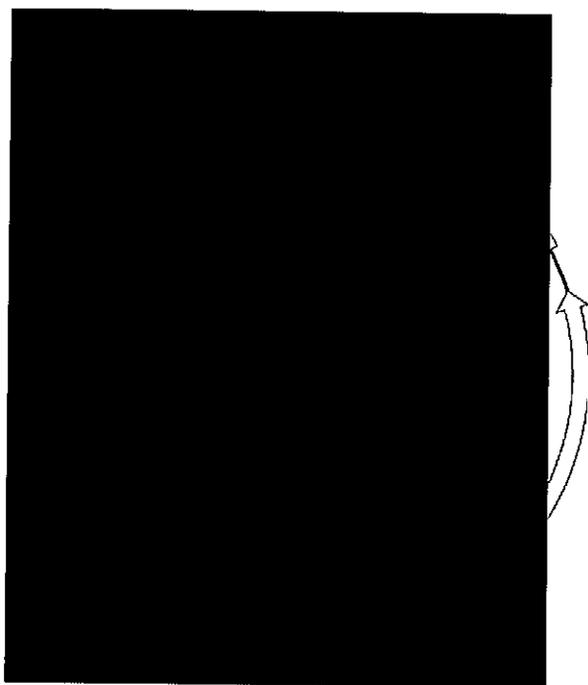


Fig. 1. Plasmid map of pEBTet. The backbone (from *oriP* clockwise to the puromycin resistance cassette) stems from pCEP-Pu (see Experimental procedures). pEBTetD (11.2 kb) lacks the bovine growth hormone poly(A) site, but is otherwise identical.

cells after transfection and antibiotic selection (not shown).

Initially we tried to partition all elements to two plasmids in order to avoid a single large plasmid. However, with the two-plasmid system, where both plasmids contained *oriP* and a different selection marker, growth of transfected cells was unsteady, perhaps because of *oriP* interference. Actually, our worries over the relatively large pEBTet vector (11.5 kb) were unfounded, as standard cloning procedures can be followed.

With pEBTet, the phase of antibiotic selection and cell expansion is much shorter than in other systems outlined above. It takes only about a week after transfection until it is possible to harvest a fully grown culture flask (175 cm²). For all subsequent analysis, pooled cells were used. With seven cDNAs coding for transport proteins, northern blot analysis of 293 cells transfected with the respective pEBTet plasmids consistently revealed strong transcription in the on-state (= 100%) and low background (1–2%, measured by radioluminography) in the off-state (Fig. 2; Table 1). We have observed comparable ratios (100 : 1) with the Flp-InTM-T-RexTM system. Thus, as far as regulation of transcription is concerned, the pEBTet vector works

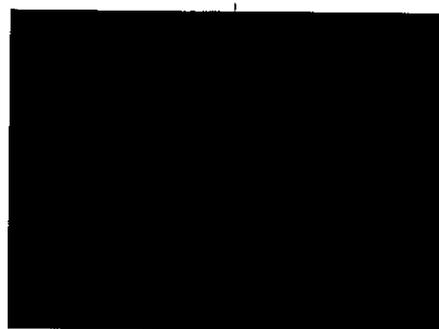


Fig. 2. Northern analysis: regulation of transcription with pEBTet/OCT1h and pEBTetD/OCT1h. mRNA was isolated from stably transfected 293 cell pools that had been cultured with or without 1 µg·mL⁻¹ doxycycline for 20 h. The RNA blot was first hybridized with a human OCT1 probe. Without stripping, the blot was then hybridized with a GAPDH probe to determine RNA loading.

well: in the off-state, *tetO2* elements are sufficiently covered by TetR to block transcription almost completely.

Transporter expression in 293 cells was assayed functionally by initial rates of uptake of substrates; initial rates of uptake are directly proportional to transporter number. With the ergothioneine transporter from human (ETTh; see Fig. 3A) and enhanced green fluorescent protein (eGFP) chimeras of ETTh and of ORCTL3h (not shown), the high signal-to-noise ratio of the mRNA corresponded to a similar ratio for transport function or eGFP fluorescence intensity. However, with OCT1h (Fig. 3B) and OCT2h (not shown) we obtained inadequate ratios; for our assays, we aim for at least a 10 : 1 ratio. It was reasoned that with some cDNAs, even the low mRNA levels in the off-state generate, because of highly efficient translation, considerable amounts of protein. A 100-fold increase in mRNA level does not increase protein in an equivalent proportion because of the limited capacity of the synthesis machinery, especially for membrane proteins [17]. To improve the signal-to-noise ratio, we thus aimed to reduce the efficiency of translation. In our first attempt, we constructed three variants of pEBTet with hairpins of graded stability ($\Delta G = -26, -33, \text{ or } -40 \text{ kcal}\cdot\text{mol}^{-1}$, calculated with MFOLD [18] version 3.2; 1 cal = 4.184 J) in the 5'-untranslated region downstream of the tandem *tetO2* element. The hairpins were intended to block ribosome progression [19]. Unfortunately, even with the most stable hairpin, the functional signal-to-noise ratio was only slightly improved (not shown).

Our second attempt was based on the notion that the number of translations per mRNA molecule may

Table 1. Regulation of transcription of transporter cDNAs with pEBTet and pEBTetD vectors. For each construct, mRNA was isolated from stably transfected 293 cell pools that had been cultured either with or without $1 \mu\text{g}\cdot\text{mL}^{-1}$ doxycycline for 20 h. mRNA was quantitated by northern analysis with radiolabelled probes followed by radioluminography. RNA blots were successively analyzed with a transporter probe and with a GAPDH probe (cf. Fig. 2). Relative background transcription was calculated from the ratios of signals for transporter mRNA and GAPDH mRNA. With OCT1, OCT2, and CAT4 both vectors were analyzed alongside on a single blot. OCT, organic cation transporter; CAT, cationic amino acid transporter.

Transporter cDNA (human)		Vector	Transporter mRNA/GAPDH mRNA		Relative background transcription (%)
Name	Gene symbol		Doxycycline +	Doxycycline -	
ETT	<i>SLC22A4</i>	pEBTet	0.014	1.4	1.0
ORCTL3	<i>SLC22A13</i>	pEBTet	0.014	1.6	0.9
FLIPT1	<i>SLC22A16</i>	pEBTet	0.0011	0.096	1.1
EMT	<i>SLC22A3</i>	pEBTet	0.0052	0.33	1.6
OCT1	<i>SLC22A1</i>	pEBTet	0.045	1.9	2.3
		pEBTetD	0.0017	0.22	0.8
OCT2	<i>SLC22A2</i>	pEBTet	0.068	3.4	2.0
		pEBTetD	0.0077	0.44	1.7
CAT4	<i>SLC7A4</i>	pEBTet	0.031	4.9	0.6
		pEBTetD	0.012	1.5	0.8

be influenced by the 3' end [20,21]. We thus deleted the bovine growth hormone polyadenylation site downstream from the cDNA of interest (Fig. 1) to generate plasmid pEBTetD (11.2 kb). The corresponding mRNA will then lack a poly(A) tail, which is a major stabilizing factor. Conversely, without a polyadenylation site and thus without endonucleolytic cleavage, the mRNA could become much longer, which would increase stability. However, it became evident from northern analysis (Fig. 2) that *oriP*, which contains 24 EBNA-1 binding sites and a 65 base dyad symmetry element [22], can function as a terminator region of RNA polymerase II. Close inspection revealed that the mRNA of OCT1h was predominantly terminated upstream of the EBNA-1 binding site region. With pEBTetD, the copy number of OCT1h mRNA was reduced as compared with pEBTet by a factor of nine in the on-state and by a factor of 26 in the off-state (Table 1).

With pEBTetD, we obtained a high functional signal-to-noise ratio for OCT1h (Fig. 3B). For ETT, regulation of expression was improved to Flp-InTM-T-RexTM system values (Fig. 3A). After 10 weeks of continuous cell culture, the on/off activity ratio was still maintained for ETT (not shown). Figure 4 shows results from analysis of eGFP expression by flow cytometry. With pEBTetD/eGFP-transfected cells the fluorescence intensity in the off-state (median 6.5) was slightly higher than autofluorescence from nontransfected cells (3.0); in the on-state, median fluorescence intensity was strongly increased (84.3; this amounts to stimulation by a factor of 23). By comparison, with pEBTet/eGFP-transfected cells, the median fluorescence intensity was 195 in the off-state and 1860 in

the on-state (this amounts to stimulation by a factor of 9.7). Thus, the background in the off-state was much lower with pEBTetD than with pEBTet. However, in contrast to expression of membrane proteins, the level of expression of cytosolic eGFP in the on-state was higher with pEBTet. It follows that pEBTetD provides low background and moderate expression levels. pEBTet offers very high expression, but the background levels, depending on the cDNA, may be intolerable.

It should be noted that with most cDNAs, dishes of pEBTet-transfected cells showed two- to three-fold reduced protein content after culture with $1 \mu\text{g}\cdot\text{mL}^{-1}$ doxycycline for 20 h versus noninduced control cells. No such differences were observed with pEBTetD. It would thus seem that the large amount of polyadenylated mRNA generated in the on-state from pEBTet can impair cell proliferation or viability.

The flow cytometry data for both pEBTet and pEBTetD show a considerable spread in fluorescence intensity; this has also been observed with other expression systems [11]. It remains to be seen whether stably transfected single cell clones can have much higher factors of inducibility than those calculated above for the pools. Bornkamm *et al.* [11] have recently presented an intricate EBV plasmid (pRTS-1; size including eGFP and luciferase cDNAs is 18.4 kb) that uses two fusion proteins, an optimized version of rtTA plus a tTS, to regulate expression from a dual tetracycline promoter. With pRTS-1 there was very high inducibility (e.g. by a factor of 140 000) for single clones in the luciferase assay, while other clones showed hardly any induction when eGFP was assayed. This suggests that for very high inducibility it may be

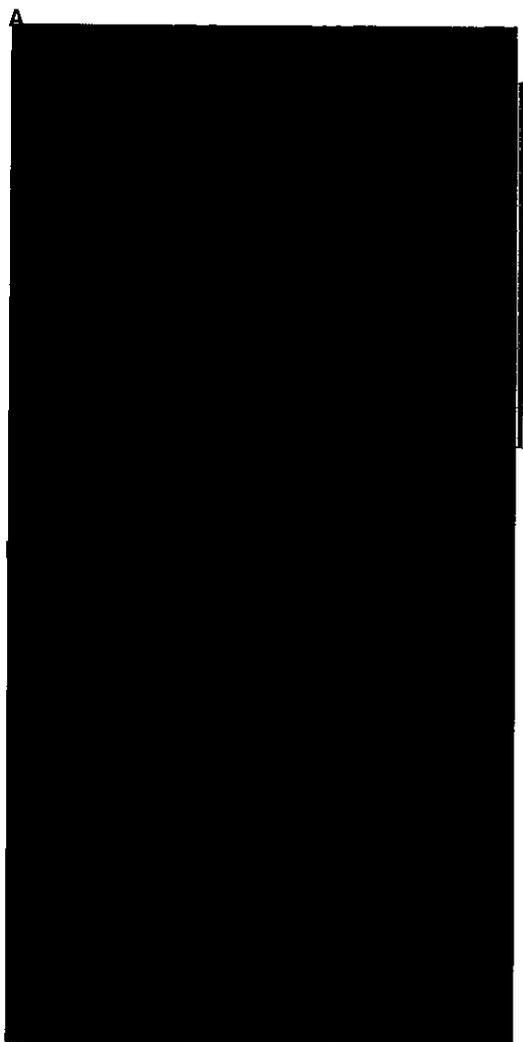


Fig. 3. Validation of pEBTet and pEBTetD vectors on the level of protein function. (A) Regulation of expression ETTh, which resides in the plasma membrane. Ergothioneine (ET) content was assayed by LC-MS/MS. The clearance equals initial rate of specific uptake (= uptake mediated by expressed carrier) divided by substrate concentration. For each of the bars, endogenous ET content was determined in parallel and subtracted from total ET content to yield the carrier-mediated increase of ET during the incubation with substrate (1 min, $10 \mu\text{mol}\cdot\text{L}^{-1}$). With nontransfected cells, no ET was detected. (B) Regulation of expression of the human organic cation transporter type 1 (OCT1h), which also resides in the plasma membrane. Transporter expression in 293 cells was assayed by initial rates of uptake of radiolabelled 1-methyl-4-phenylpyridinium (MPP⁺). Uptake was measured by liquid scintillation counting. Non-specific uptake into nontransfected cells due to diffusion, endocytosis, or binding was subtracted from total uptake to yield the carrier-mediated uptake of MPP⁺ (1 min, $0.1 \mu\text{mol}\cdot\text{L}^{-1}$) as shown.

necessary to perform clonal selection also with EBV vectors. However, clonal selection eliminates the main benefit of EBV vectors, i.e. to save set-up time. Our

emphasis was therefore on the analysis of cell pools. We do not assume that our system is superior in terms of inducibility of single clones; moreover, as with other systems, stability over culture time is probably limited [11]. However, our results for pEBTetD cell pools in continuous culture up to 10 weeks indicate useful factors of inducibility on the level of protein function. Clearly, in many situations, e.g. in RNA interference experiments, an activity ratio of 10 : 1 is sufficient. Importantly and in contrast to pRTS-1, our vector is based on the simple TetR; the use of chimeric transcription factor domains with the inherent risk of multiple effects on gene expression is avoided.

In summary, we have developed plasmid pEBTetD for full establishment of doxycycline-inducible protein expression in human cell lines by just a single transfection. For closely matching cellular backgrounds we use continuous expression of the original TetR instead of TetR-transactivator fusion proteins. As clonal isolation is unnecessary and because of efficient episomal propagation of the Epstein-Barr vector, our approach saves 2–10 weeks of time.

Experimental procedures

Plasmid constructs

All constructs were assembled by standard cloning methods and confirmed by DNA sequencing. The backbone of pEBTet stems from pCEP-Pu [23]. The *Tn10*-derived TetR open reading frame and the *CMV-tetO2-tetO2* promoter were taken from pcDNA6/TR and pcDNA5/FRT/TO (Invitrogen, Karlsruhe, Germany), respectively. The nucleotide sequence of plasmid pEBTet (11 486 bases) is available online. pEBTetD (11 200 b) corresponds to pEBTet except for the bovine growth hormone polyadenylation site deletion region: CTCGAG CGATCGCGGC CGCGGGG (original pEBTet sequence underlined). cDNAs were inserted into the polylinkers of pEBTet or pEBTetD. The cDNA sequence of ETTh [9] corresponds to GenBank entry Y09881. For pEBTet/ETTh, the 5' interface between cDNA and pEBTet is AAGCTT GAATTCTGCAGAT TCGA gccacc ATGCGGGA (polylinker in bold, Kozak motif in lower case, cDNA underlined); the 3' interface is ATTTCTAGA TCCAGCAC. For pEBTetD/ETTh, the 5' interface is identical; the 3' interface is ATTTCTAGA TCCAGCACAGTG GCGGCCGCGG. Our cDNA of OCT1h [24] corresponds to GenBank entry X98332 except for 2 bases (228C > T and 1294A > G). For pEBTet/OCT1h, the 5' interface is TCGGATCC gccacc ATG CCCAC; the 3' interface is CTTCGCAG CTCGAGTC. For pEBTetD/OCT1h, both interfaces are identical. Our cDNA of the *SLC22A16* gene corresponds to GenBank entry NM_033125.2 except for two bases (244T > C and



Fig. 4. Analysis of eGFP expression in 293 cells by flow cytometry. 293 cell pools stably transfected with either pEBTet/eGFP or pEBTetD/eGFP were cultured with or without $1 \mu\text{g}\cdot\text{mL}^{-1}$ doxycycline for 20 h, resuspended, and then analyzed for eGFP fluorescence by flow cytometry. The fluorescence recorded for untransfected control cells corresponds to autofluorescence. Arithmetic means of fluorescence intensity were 3.4 (untransfected cells), 26 (pEBTetD/eGFP, off-state), 400 (pEBTetD/eGFP, on-state), 330 (pEBTet/eGFP, off-state), and 2700 (pEBTet/eGFP, on-state).

1293T > C) (D. Kroppeit, R. Berkels & D. Gründemann unpublished results). For pEBTet/SLC22A16h, the 5' interface is GGTACC CCCCCGGA; the 3' interface is ATGCCCTGC GGGGATCCAC TAGTAACGGC CGCC AGTGTG CTGGAATTCT GCAGATATCC ATCACAC TGGCGGCC. The cDNA of eGFP corresponds to GenBank entry U57609. For pEBTet/eGFP, the 5' interface is GGTACCG CGGGCCCGGGATCCATC gccacc ATGG TGA; the 3' interface is CAAGTAAA GCGGCCGC. For pEBTetD/eGFP, the 5' interface is identical; the 3' interface is CAAGTAAA GCGGCCCGCGG.

Cell culture, transfection, and flow cytometry

293 cells (ATCC CRL-1573), a transformed cell line derived from human embryonic kidney, were grown at 37 °C in a humidified atmosphere (5% CO₂) in plastic culture flasks (Falcon 3112, Becton Dickinson, Heidelberg, Germany). The growth medium was Dulbecco's modified Eagle medium (Life Technologies 31885-023, Invitrogen) supplemented with 10% fetal bovine serum (PAA Laboratories, Cölbe, Germany). Medium was changed every 2–3 days and the culture was split every 5 days.

Cells were transfected with supercoiled plasmid DNA by lipofection with the Tfx-50 reagent according to the protocol of the vendor (Promega, Mannheim, Germany). From the next day on, stably transfected cells were selected with $3 \mu\text{g}\cdot\text{mL}^{-1}$ puromycin (PAA Laboratories); puromycin was always present in subsequent cell culture to ascertain plasmid maintenance. To turn on protein expression, cells were cultivated for at least 20 h in regular growth medium supplemented with $1 \mu\text{g}\cdot\text{mL}^{-1}$ doxycycline (195044, MP Bio-medicals, Eschwege, Germany). For flow cytometry, cells were resuspended in growth medium and analysed on a FACSCalibur flow cytometer using CELLQUEST PRO software (BD Biosciences, San Jose, CA, USA).

Transport assays

For measurement of uptake of radiolabelled 1-methyl-4-phenylpyridinium (MPP⁺), cells were grown in surface culture on 60 mm polystyrol dishes (Nuncion 150288, Nunc, Roskilde, Denmark) precoated with $0.1 \text{ g}\cdot\text{L}^{-1}$ poly L-ornithine in 0.15 M boric acid–NaOH, pH 8.4. Cells were used for uptake experiments at a confluence of at least 70%. Uptake was measured at 37 °C. After preincubation for at least 20 min in 4 mL of uptake buffer (in $\text{mmol}\cdot\text{L}^{-1}$: 125 NaCl, 25 Hepes–NaOH pH 7.4, 5.6 (+)glucose, 4.8 KCl, 1.2 KH₂PO₄, 1.2 CaCl₂, 1.2 MgSO₄), the buffer was replaced with 3 mL of [³H]MPP⁺ (at $0.1 \mu\text{mol}\cdot\text{L}^{-1}$) in uptake buffer. Incubation was stopped after 1 min by rinsing the cells four times each with 4 mL ice-cold uptake buffer. Subsequently, the cells were solubilized with 0.1% v/v Triton X-100 in 5 $\text{mmol}\cdot\text{L}^{-1}$ Tris–HCl pH 7.4, and radioactivity was determined by liquid scintillation counting.

Uptake of ergothioneine ($10 \mu\text{mol}\cdot\text{L}^{-1}$) was determined by LC-ESI-MS/MS. Cells were assayed and washed as above, solubilized with $4 \text{ mmol}\cdot\text{L}^{-1}$ HClO₄ and stored at –20 °C. After centrifugation (1 min, 16 000 g, 20 °C) of the thawed lysates, 100 μL of the supernatant was mixed with 10 μL unlabelled MPP⁺ iodide ($5.0 \text{ ng}\cdot\mu\text{L}^{-1}$), which served as the internal standard. Of this mixture, 20 μL samples were analyzed by LC-MS/MS on a triple quadrupole mass spectrometer (TSQ Quantum, Thermo Electron, Dreieich, Germany). Atmospheric pressure ionization with positive electrospray was used. The LC system consisted of Surveyor LC-pump, autosampler, and Waters Atlantis HILIC silica column (length 100 mm, diameter 3 mm, particle size 5 μm). The solvent for isocratic chromatography (flow rate $250 \mu\text{L}\cdot\text{min}^{-1}$) was made of methanol (70%) and 0.1% formic acid (30%). For quantification of ergothioneine by selected reaction monitoring, m/z 230 and

m/z 127 were selected as parent and fragment, respectively (collision energy: 24 V; scan time: 0.3 s). The area of the intensity versus time peak was integrated and divided by the area of the MPP^+ peak to yield the analyte response ratio. Linear calibration curves ($R^2 > 0.99$) were constructed from at least six standards which were prepared using control cell lysates as solvent. Sample analyte content was calculated from the analyte response ratio and the slope of the calibration curve, obtained by weighted linear regression.

For radiotracer assays, protein was measured by the bicinchoninic acid assay [25] with bovine serum albumin as standard. The protein content of MS samples was estimated from the response ratio for proline, which was calibrated against the bicinchoninic acid assay (4–6 matched cell dishes) for each MS session.

Northern blot analysis

Northern analysis was performed with ^{33}P -labelled double-stranded DNA probes essentially as described in [26]. Radioactivity was quantitated with a Fujifilm BAS-1800 II analyzer. Transcripts were normalized by reference to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels.

Calculations

Arithmetic means ($n = 3$) are given with SEM.

Drugs

Radiotracers used were as follows: MPP^+ iodide (H-3, 2.2 kBq·pmol $^{-1}$, ART-150, ARC, St Louis, MO, USA).

Unlabeled compounds used were as follows: MPP^+ iodide (D-048, Sigma-Aldrich, Munich, Germany), L-(+)-ergothioneine (F-3455, Bachem, Bubendorf, Switzerland). All other chemicals were at least of analytical grade.

Acknowledgements

Supported by Deutsche Forschungsgemeinschaft (GR 1681/2–1). We thank B. Steinrücken, S. Kalis and R. Baucks for skilful technical assistance, and N. Smyth for providing pCEP-Pu.

References

- 1 Tate CG, Haase J, Baker C, Boorsma M, Magnani F, Vallis Y & Williams DC (2003) Comparison of seven different heterologous protein expression systems for the production of the serotonin transporter. *Biochim Biophys Acta* **1610**, 141–153.
- 2 Gossen M & Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA* **89**, 5547–5551.
- 3 Berens C & Hillen W (2003) Gene regulation by tetracyclines. Constraints of resistance regulation in bacteria shape TetR for application in eukaryotes. *Eur J Biochem* **270**, 3109–3121.
- 4 Leight ER & Sugden B (2001) Establishment of an oriP replicon is dependent upon an infrequent, epigenetic event. *Mol Cell Biol* **21**, 4149–4161.
- 5 Yates JL, Warren N & Sugden B (1985) Stable replication of plasmids derived from Epstein–Barr virus in various mammalian cells. *Nature* **313**, 812–815.
- 6 Lupton S & Levine AJ (1985) Mapping genetic elements of Epstein–Barr virus that facilitate extrachromosomal persistence of Epstein–Barr virus-derived plasmids in human cells. *Mol Cell Biol* **5**, 2533–2542.
- 7 Young JM, Cheadle C, Foulke JS Jr, Drohan WN & Sarver N (1988) Utilization of an Epstein–Barr virus replicon as a eukaryotic expression vector. *Gene* **62**, 171–185.
- 8 Sclementi CR, Baba EJ & Calos MP (2000) An extrachromosomal tetracycline-regulatable system for mammalian cells. *Nucleic Acids Res* **28**, E80.
- 9 Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A & Schömig E (2005) Discovery of the ergothioneine transporter. *Proc Natl Acad Sci USA* **102**, 5256–5261.
- 10 Lang Z & Feingold JM (1996) An autonomously replicating eukaryotic expression vector with a tetracycline-responsive promoter. *Gene* **168**, 169–171.
- 11 Bornkamm GW, Berens C, Kuklik-Roos C, Bechet JM, Laux G, Bachl J, Korndoerfer M, Schlee M, Holzel M, Malamoussi A *et al.* (2005) Stringent doxycycline-dependent control of gene activities using an episomal one-vector system. *Nucleic Acids Res* **33**, e137.
- 12 Baron U, Gossen M & Bujard H (1997) Tetracycline-controlled transcription in eukaryotes: novel transactivators with graded transactivation potential. *Nucleic Acids Res* **25**, 2723–2729.
- 13 Akagi K, Kanai M, Saya H, Kozu T & Berns A (2001) A novel tetracycline-dependent transactivator with E2F4 transcriptional activation domain. *Nucleic Acids Res* **29**, E23.
- 14 Ivanova L, Brandli J, Saudan P & Bachmann MF (2005) Hybrid Sindbis/Epstein–Barr virus episomal expression vector for inducible production of proteins. *Biotechniques* **39**, 209–212.
- 15 Yao F, Svensjö T, Winkler T, Lu M, Eriksson C & Eriksson E (1998) Tetracycline repressor, tetR, rather than the tetR-mammalian cell transcription factor fusion derivatives, regulates inducible gene expression in mammalian cells. *Hum Gene Ther* **9**, 1939–1950.
- 16 Wishart JA, Hayes A, Wardleworth L, Zhang N & Oliver SG (2005) Doxycycline, the drug used to control the tet-regulatable promoter system, has no effect on global gene expression in *Saccharomyces cerevisiae*. *Yeast* **22**, 565–569.

- 17 Grishammer R (2006) Understanding recombinant expression of membrane proteins. *Curr Opin Biotechnol* **17**, 337–340.
- 18 Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**, 3406–3415.
- 19 Kozak M (1989) Circumstances and mechanisms of inhibition of translation by secondary structure in eucaryotic mRNAs. *Mol Cell Biol* **9**, 5134–5142.
- 20 Kahvejian A, Svitkin YV, Sukarieh R, M'Boutchou MN & Sonenberg N (2005) Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes Dev* **19**, 104–113.
- 21 Kozak M (2004) How strong is the case for regulation of the initiation step of translation by elements at the 3' end of eukaryotic mRNAs? *Gene* **343**, 41–54.
- 22 Yates JL, Camiolo SM & Bashaw JM (2000) The minimal replicator of Epstein–Barr virus oriP. *J Virol* **74**, 4512–4522.
- 23 Kohfeldt E, Maurer P, Vannahme C & Timpl R (1997) Properties of the extracellular calcium binding module of the proteoglycan testican. *FEBS Lett* **414**, 557–561.
- 24 Gründemann D, Hahne C, Berkels R & Schömig E (2003) Agmatine is efficiently transported by non-neuronal monoamine transporters EMT and OCT2. *J Pharmacol Exp Ther* **304**, 810–817.
- 25 Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ & Klenk DC (1985) Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**, 76–85.
- 26 Schömig E, Spitzenberger F, Engelhardt M, Martel F, Örding N & Gründemann D (1998) Molecular cloning and characterization of two novel transport proteins from rat kidney. *FEBS Lett* **425**, 79–86.



CellLight® Mitochondria-RFP, BacMam 2.0

(Molecular Probes®)

Description

CellLight® Mitochondria-RFP is a modified insect virus (baculovirus) expressing a fusion construct of a mitochondrial marker and red fluorescent protein. CellLight® reagents combine the utility and selectivity of fluorescent proteins with the transduction efficiency of BacMam technology, enabling unambiguous staining of organelles, cellular structures, and processes in live mammalian cells. They are provided in a ready-to-use format—simply add, incubate, and image—with highly efficient expression in cell lines, primary cells, stem cells, and neurons.

CellLight® Mitochondria-RFP is:

- **Highly Efficient:** >90% transduction of a wide range of mammalian cell lines, including primary cells, stem cells, and neurons
- **Fast and Convenient:** Simply add CellLight® Mitochondria-RFP reagent to your cells, incubate overnight, and image – or store frozen, assay-ready cells for later use
- **Safe:** CellLight reagents are non-replicating in mammalian cells, lack of observable

cytopathic effect, and are suitable for biosafety level (BSL) 1 handling

- **Flexible:** You can co-transduce more than one BacMam reagent for multiplex experiments or co-localization studies and tightly control expression levels by just varying the dose

CellLight® Reagents Principle

CellLight® reagents are fusion constructs of signal peptides or cell structure proteins with premiere emGFP, TagRFP or CFP for accurate and specific targeting to sub-cellular compartments and structures. A variety of targets, including cytoskeleton, mitochondria, and secretory compartments, are available for convenient multiplexing, co-localization studies, and imaging of dynamic cellular processes where high spatial and temporal resolution is required. The CellLight® reagents tolerate fixation with formaldehyde and are therefore compatible with fixed-cell analysis.

BacMam Technology

The BacMam technology is based on an insect virus (baculovirus) for efficient transduction and transient expression in mammalian cells. The baculovirus has been modified to include an expression cassette containing the CellLight® fusion construct.

BacMam 2.0 incorporates elements that help greatly enhance transduction efficiency and expression levels: a pseudotyped capsid protein for more efficient cell entry, an enhanced CMV promoter, and the Woodchuck hepatitis post-transcriptional regulatory element (WPRE).

Baculoviruses do not replicate in mammalian cells and thus have an excellent safety profile and lack cytopathic effects on cells.

Work Flow Convenience

Unlike expression vectors, BacMam reagents enable titratable and reproducible transient protein expression. There is no need for harsh transfection methods or tedious cloning. To achieve highly efficient expression even in sensitive cells, such as stem cells, neurons, and primary cells, just add CellLight® reagents to cells in complete medium, incubate, and image the next day. Alternatively, mix CellLight® reagent and cells at the time of plating.

Co-transduction efficiencies are high allowing multiple CellLight® reagents to be readily used in the same experiment when multiple structures or pathways need to be labeled. CellLight® reagents also tolerate fixation with formaldehyde and are thus compatible with antibody-based fixed-cell analysis.

Typically, transiently transduced cells express fusion protein for about five days, though in slowly dividing cells, such as some primary cell types, expression has been demonstrated for up to two weeks; in terminally differentiated neurons we have recorded images more than three weeks after

Catalog Number

C10601

Size

1 ml

List Price

(CAD) 465.00

Related Products

We offer a range of BacMam-based reagents beyond CellLight® reagents, including the [BacMam GFP Transduction Control](#) that is ideal to test out the technology and optimize transduction conditions, Promo™ Biosensors, including [Promo™ Autophagy Sensor](#), ion channel drug targets, pathway analysis kits, and more.

[Learn more about these products and the BacMam technology](#)

[See other imaging tools and reagents](#)

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Regulatory Statement: For Research Use Only. Not for any animal or human therapeutic or diagnostic use.

[CellLight Reagents *BacMam 2.0* Quick Reference](#)

Color: Red-Orange, Orange

[CellLight Reagents *BacMam 2.0*](#)

Quantity: 1 vial

Label or Dye: RFP (TagRFP)

Emission Class: Visible

Excitation Class: Visible

Flow Cytometer Laser Lines: 532

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : **Cholera Toxin Vibrio cholerae**

Product Number : C8052
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA
Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300

Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Bowel

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects Moderate skin irritant
Moderate eye irritant

GHS Classification

Acute toxicity, Oral (Category 4)
Acute toxicity, Dermal (Category 4)
Skin irritation (Category 3)
Acute aquatic toxicity (Category 3)
Chronic aquatic toxicity (Category 3)

GHS Label elements, including precautionary statements

Pictogram



Signal word Warning

Hazard statement(s)

H302 + H312 Harmful if swallowed or in contact with skin.
H316 Causes mild skin irritation.
H412 Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P273 Avoid release to the environment.
P280 Wear protective gloves/ protective clothing.

HMIS Classification

Health hazard: 2
Chronic Health Hazard: *

Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. Causes respiratory tract irritation.
Skin Harmful if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion Harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cholera enterotoxin
Cholergen

CAS-No.	EC-No.	Index-No.	Concentration
Tris (hydroxymethyl) aminomethane			
77-86-1	201-064-4	-	>= 5.82 - <= 5.94 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	>= 31.3 - <= 31.9 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	>= 1.83 - <= 1.87 %
Sodium chloride			
7647-14-5	231-598-3	-	>= 57.6 - <= 58.8 %
Exotoxin, vibrio cholerae			
9012-63-9	-	-	>= 0.5 - <= 2.5 %
Sodium azide			
26628-22-8	247-852-1	011-004-00-7	>= 0.96 - <= 0.98 %

4. FIRST AID MEASURES

General advice

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

If inhaled

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

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

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

5. FIREFIGHTING MEASURES

Conditions of flammability

Not flammable or combustible.

Suitable extinguishing media

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

Special protective equipment for firefighters

Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides

Explosion data - sensitivity to mechanical impact

no data available

Explosion data - sensitivity to static discharge

no data available

6. ACCIDENTAL RELEASE MEASURES**Personal precautions**

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up

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

7. HANDLING AND STORAGE**Precautions for safe handling**

Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment**Respiratory protection**

For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator. For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

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

Eye protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

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

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls

Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

no data available

Conditions to avoid

no data available

Materials to avoid

Dimethyl sulfate, Acid chlorides, Halogenated hydrocarbon, Metals, Acids

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides

Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50

no data available

Inhalation LC50

no data available

Dermal LD50

no data available

Other information on acute toxicity

no data available

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

Eyes: no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

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

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)

no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)

no data available

Aspiration hazard

no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Harmful if swallowed.
Skin	Harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Signs and Symptoms of Exposure

Laboratory experiments in animals have shown sodium azide to produce a profound hypotensive effect, demyelination of myelinated nerve fibers in the central nervous system, testicular damage, blindness, attacks of rigidity, and hepatic and cerebral effects.

Synergistic effects

no data available

Additional Information

RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability

no data available

Bioaccumulative potential

no data available

Mobility in soil

no data available

PBT and vPvB assessment

no data available

Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Harmful to aquatic life with long lasting effects.

13. DISPOSAL CONSIDERATIONS**Product**

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION**DOT (US)**

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

15. REGULATORY INFORMATION**WHMIS Classification**

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant
		Moderate eye irritant

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

16. OTHER INFORMATION**Text of H-code(s) and R-phrase(s) mentioned in Section 3****Further information**

Copyright 2011 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.



TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	0.1 µg
Proposed Storage Dose:	1000 µg
LD₅₀ (species):	250 µg

Calculation:	
250 µg/kg	x 50 kg/person
Dose per person based on LD ₅₀ in µg = 12500	
LD₅₀ per person with safety factor of 10 based on LD₅₀ in µg =	1250

Comments/Recommendations:

Note from e-mail by Cindy Shao (03/29/2012) - 1mL medium is added to 100 ng toxin

**Pathogen Regulation Directorate
Direction de la réglementation
des agents pathogènes**



Public Health Agence de la santé
Agency of Canada publique du Canada

WHO Collaborating
Centre for Biosafety



Centre collaborateur OMS
pour les techniques de biosécurité

100 chemin Colonnade Road, Loc.: 6201A
Ottawa, Ontario, Canada K1A 0K9

Tel: (613) 957-1779 Fax: (613) 941-0596

TO/À: Dale Laird

DATE:

FEBRUARY 20, 2012

University of Western Ontario

FAX: 519-661-3420

TEL: 519-661-2111
ext:86827

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*** COMMENTS - COMMENTAIRES ***

Please find attached a copy of your '**Compliance Letter**' concerning the purchasing of biological material from a Canadian distributor. The original "Letter" is being sent to you through regular mail.

Vous trouverez sous pli une copie de votre '**Lettre de conformité**' concernant l'achat de matières biologiques chez un distributeur Canadien. La copie originale de votre "Lettre" vous parviendra par la poste.

Be advised, however, that if these products contain matter of animal origin (such as bovine serum, etc.) or animal pathogens, you will need to contact the Canadian Food Inspection Agency (CFIA) at (613) 221-7068 for their consideration.

Veuillez noter, cependant, que si ces produits contiennent des substances d'origine animale (par exemple du serum bovin) ou des pathogènes animaux, vous devez contacter l'Agence canadienne d'inspection des aliments (ACIA) au (613) 221-7068 afin d'obtenir leur approbation.

Thank you.

Merci



Public Health
Agency of Canada

Agence de la santé
publique du Canada

Compliance Letter No. | N° de lettre de conformité: C-12-1146
Expiry Date | Date d'expiration: 2014-02-09

Compliance Letter

This letter serves to confirm that the Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist based on the requirements identified in the Laboratory Biosafety Guidelines, 3rd Ed., 2004 for the facility identified below, and found the information submitted acceptable.

Lettre de Conformité

Par la présente, la Direction de la réglementation des agents pathogènes confirme qu'elle a examiné la liste de contrôle de niveau de confinement 2 en vertu des exigences des Lignes directrices en matière de biosécurité en laboratoire, 3e édition, 2004 pour l'installation identifiée ci-dessous et a déterminé que l'information fournie est acceptable.

HPTA Registration No. | N° d'enregistrement en vertu de la LAPHT:
R-06-000598

Entity / Facility | Organisation / Installation:
University of Western Ontario
Department of Anatomy & Cell Biology

Attention | À l'attention de:
Dale Laird

Address | Adresse:
Dental Science Building
1151 Richmond Street
London, ON
N6A 5C1

Laboratory Room No(s). | N° du/des pièce(s) du/des laboratoire(s):
00076A.

Type of work | Description du travail:
in vitro only | *in vitro* seulement

Should you have any questions regarding this letter, please do not hesitate to contact our office:
permit-permis@phac-aspc.gc.ca or 613-957-1779.

Pour toutes questions concernant cette lettre, n'hésitez pas à nous contacter : permit-permis@phac-aspc.gc.ca ou 613-957-1779.

Geneviève Lacroix

Lead Biosafety Inspector |
Inspecteur / Inspectrice principal(e) en biosécurité

FEBRUARY 20, 2012

Date



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

Product Information

Cholera Toxin from *Vibrio cholerae*

Catalog Number **C8052**

Storage Temperature 2–8 °C

CAS RN 9012-63-9

Synonyms: Cholera enterotoxin; Cholera toxin

Product Description

Cholera toxin is the virulent factor from *Vibrio cholerae* that leads to severe diarrhea followed by dehydration in humans.^{1,2} Several bacterial toxins are ADP-ribosyltransferases with protein substrates. Many of the substrates ADP-ribosylated by bacterial protein toxins are G-proteins, which are involved in signal transduction and ADP-ribosylation is one of the more significant post translational modifications of proteins. The ADP-ribosylation activity of cholera toxin activates adenylate cyclase, resulting in the production of cyclic AMP by adenylate cyclase, which causes many metabolic alterations.^{1,2}

Cholera toxin belongs to the AB₅-subunit family of toxins.¹ The native hexameric protein has a molecular mass of ~85 kDa and contains two subunits. It consists of a single A subunit (~27.2 kDa), responsible for the ADP-ribosylation activity, and five B subunits (~11.6 kDa each), which are arranged as a pentameric ring with an apparent 5-fold symmetry and are associated with the cell surface receptor binding and subsequent internalization (transmembrane transport) of the enzymatic component.^{3,4}

A single isoelectric variant of the cholera toxin has been isolated, which crystallizes readily and reproducibly.⁵ Cholera toxin has an isoelectric point (pI) of 6.6. Chromatographic properties, however, suggest a cationic surface is exposed at pH 7.0, which apparently resides in B subunit.⁶

The entire hexameric complex is required for toxic behaviour. Cholera toxin, the intact pentamer of B subunits, interacts with a ganglioside G_{M1} membrane receptor, but cannot activate adenylate cyclase; whereas, the A subunit alone does not enter the cell.⁷

Due to the effect on adenylate cyclase, cholera toxin and its purified A subunit are frequently used for the study of signal transduction mechanisms. In addition, cholera toxin acts as an adjuvant through the stimulation of B lymphocytes.

The cholera toxin B subunit alone is used for tracking in neurological research, taking advantage of G_{M1} ganglioside binding and retrograde transport. Tissue culture cells treated with cholera toxin are not killed and tissues of animals do not become necrotic.

The B subunit is non-toxic to cells and possesses no intrinsic adenylate cyclase activity. The cholera toxin B subunit (CTB) attaches to cells by binding to ganglioside G_{M1}.⁸ As a result, it has been shown to be a good label for microglial cells (due to the enrichment of ganglioside G_{M1} on their cell surface), but not for oligodendrocytes or astrocytes.⁹ The B subunit has been reported to be an excellent tracer for the study of axonal transport using immunohistochemical methods. Recently it has been widely used as a marker of membrane lipid rafts, which are membrane microdomains enriched with cholesterol and sphingolipids. These lipid rafts have an important role in cell signaling and protein trafficking.¹⁰

This product is the active, native cholera toxin (composed of the A and the B subunits). It is a lyophilized powder containing ~5% protein (Lowry-TCA). When reconstituted with water to a final concentration of 1 mg cholera toxin per ml, the solution will contain 0.05 M Tris buffer salts, pH 7.5, 0.2 M NaCl, 3 mM NaN₃, and 1 mM sodium EDTA.

Purity: ~95% (SDS-PAGE)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions

Cholera toxin is soluble in water at a concentration of 10 mg/ml. Swirl bottles gently during reconstitution. Avoid vigorous pipetting of solutions that may lead to foaming. Solutions can be filtered through a 0.2 µm filter.

Storage/Stability

The product was prepared and packaged using aseptic technique and sealed under vacuum. Store the lyophilized powder and reconstituted solutions at 2–8 °C.

The product, as supplied, is stable 3 years when stored properly.

Solutions are reported to be stable for 1 year when stored at 2-8 °C and will lose biological activity after prolonged exposure to pH below 6 or above 8.⁶
DO NOT FREEZE.

References

1. Lencer, W.I., and Tsai, B., The intracellular voyage of cholera toxin : going retro. *Trends biochem. Sci.*, **128**, 639-645 (2003).
2. Finkelstein, R.A., and Dorner, F., Cholera enterotoxin (Choleraagen)., *Pharmac. Ther.*, **27**, 37-47 (1985).
3. Roda, L.G., *et al.*, Heterogeneity of purified cholera toxin. *Biochim. Biophys. Acta*, **492(2)**, 303-315 (1977).
4. Ribi, H.O., *et al.*, Three-dimensional structure of cholera toxin penetrating a lipid membrane. *Science*, **239(4845)**, 1272-1276 (1988).
5. Spangler, B.D., and Westbrook, E.M., Crystallization of isoelectrically homogeneous cholera toxin. *Biochem.*, **28**, 1333 (1989).
6. Mekalanos, J.J., *et al.*, *Meth. Enzymology*, **165**, 169-175 (1988).
7. Middlebrook, J.L., and Dorland, R.B., Bacterial toxins: cellular mechanisms of action. *Microbiol. Rev.*, **48**, 199 (1984).
8. Heyningen, S. Van, Cholera toxin: interaction of subunits with ganglioside G_{M1}. *Science*, **183**, 656-657 (1974).
9. Nedelkoska, L., and Benjamins, J.A., Binding of cholera toxin B subunit: a surface marker for murine microglia but not oligodendrocytes or astrocytes. *J. Neurosci. Res.*, **53**, 605-612 (1998).
10. Janes, P.W., *et al.*, Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. *J. Cell Biol.*, **147**, 447-461 (1999).

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