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

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

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

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

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

PRINCIPAL INVESTIGATOR (S): TING Y LEE / Lisa M. Hoffman (see below)
DEPARTMENT: UWO/SCHULICH / ROBARTS + LAWSON IMAGING
ADDRESS: RM 12003 RRI
PHONE NUMBER: 24131 pager 15131
EMERGENCY PHONE NUMBER(S):
EMAIL: tlee@imaging.robarts.ca

Location of experimental work to be carried out: Building(s) LHRI Room(s) F4-127a, F5-104, B5-251, C0-232

*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:
GRANT TITLE(S):

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

Name	UWO E-mail Address	Date of Biosafety Training
~~Dr Lisa Hoffman~~ - moved to Co-PI		
Jennifer Hadway	jhadway@uwo.ca	
Lise Desjardins	ldesjar5@uwo.ca	
Laura Morrison	lmorri67@uwo.ca	
Timothy Yeung	timothy.yeung@lhsc.on.ca	
Joo Ho Tai	jtai@imaging.robarts.ca	
Errol Stewart		
Astrid Chamson-Reig	achamson@lawsonimaging.ca	
~~Brenda Zaidi~~		
Kelly Gutpell	Kgutpell@uwo.ca	

L. Hoffman
Imaging, LHRI
email: lhoffman@lawsonimaging.ca

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

See attached sheets:

Note: multiple projects are in use, so brief descriptions of the agents; how they're used, stored & disposed are included on a summary sheet. If required, additional information on the individual research projects can be provided

Please include a one page research summary or teaching protocol.

2.0 Cell Culture
2.2 Primary cells:

Human: MDA-MB-435. From the Koropatnick lab, derived from human breast carcinoma, obtained from Dr. Donna Goldhawk at Lawson. This line is to be used to demonstrate proof-of-principle that MagA-expressing cells (line engineered by D. Goldhawk) migrate in vitro in a specified magnetic field.

Rodent: Muscle satellite cells (SCs) harvested from our transgenic murine line housed at LHRI, not a biohazard.

Other (Rabbit): VX2 rabbit papilloma virus – is a rabbit tumor that we maintain in the rabbit thigh muscle and then every 3 – 6 weeks we euthanize the rabbit take out small pieces of tumor and mix it with HBSS and inject into a new rabbit thigh and (usually) into the liver (separate protocol) of 2 rabbits on the same day for a liver tumor study. We do also maintain small pieces in -80C freezer but it does not grow as fast or as reliably from the freezer. The rabbits are incinerated and the remaining sample that is not used goes into biohazardous waste for autoclaving before disposal.

2.3 Established cells:

Human:

(1) LoVo – ATCC # CCL-229 derived from colorectal adenocarcinoma metastatic site, ATCC biosafety level 1. This line is injected into the neck/shoulder region of nude rats and then imaged for several weeks after. The rat is sent for incineration at the end of the study and the tumor is usually removed for histology and the remainder not used is disposed of in biohazardous waste.

(2) PC3 cells - ATCC CRL-1435 human prostate tumor, ATCC biosafety level 1. This line is injected into the neck/shoulder region of nude mice for imaging purposes. Again at the end of the study the tumor is removed and the mouse sent for incineration and the tumor kept for histology purposes.

(3) NCI – H1299 – ATCC # CRL-5803 Human non-small cell lung carcinoma, ATCC biosafety level 1. This is injected into the lungs mixed with matrigel in nude rats, tumor removed at end of study for histology and rat incinerated, histology done on tumor.

(4) HT-29 – ATCC # HTB-38 derived from colorectal adenocarcinoma, ATCC level 1, this line is injected into the neck/shoulder region of nude rats and then imaged for several weeks after. The rat is sent for incineration at the end of the study and the tumor is usually removed for histology and the remainder not used is disposed of in biohazardous waste.

(5) HEK (also referred to as 293) derived from human kidney, received from Dr. Greg Dekaban (RRI, UWO). Also available from InVitrogen (see attached sheets). Level 2 containment.

(6) 293T – derived from human kidney (see attached InVitrogen sheets) Level 2 containment.

Rodent:

(1) C2C12 cells - ATCC # CRL-1722, mouse muscle myoblast, ATCC biosafety level 1. This line is radiolabelled with a PET substrate, then injected intramuscularly into the hindlimbs and imaged on a uPET scanner. Following scans, muscle is removed for ex vivo analyses/histology, and the carcass disposed of in biohazardous waste.

(2) C6-glioma - ATCC # CCL-107 rat brain tumor, ATCC biosafety level 1. This is stereotactically injected into the brain of rats and imaging and then radiotherapy done. Brain is removed at end of study for histology and rat is incinerated.

(3) H9-C2 – ATCC # CRL-1446, rat cardiomyoblast line, obtained from Dr. Frank Prato's group at Lawson, ATCC biosafety level 1. This line is radiolabelled with a PET substrate, then injected intramuscularly into the heart and imaged on a uPET scanner. Following scans,muscle is removed for ex vivo analyses/histology, and the carcass disposed of in biohazardous waste.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0
- cardiotoxin (see attached sheets)
Do you use microorganisms that require a permit from the CFIA? ✓YES O NO
If YES, please give the name of the species. lentivirus
What is the origin of the microorganism(s)? Infinitrogen inte
Please describe the risk (if any) of escape and how this will be mitigated: see attached
Rooms in which lentivirus are used are Level 2+ (Hepa-certified BSCs, portable autoclave, gloves, lab coats, bleach, etc
Please attach the CFIA permit.
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

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

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? (see attached sheets) ✓YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	✓Yes O No	MDA-MB-435	Not applicable
Rodent	✓Yes O No	muscle satellite cells (mouse)	2010-067
Non-human primate	O Yes ✗ No		
Other (specify)	✓Yes O No rabbit	VX2 papilloma virus	

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

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	O Yes O No			
Rodent	O Yes O No			
Non-human primate	O Yes O No			
Other (specify)	O Yes O 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 O 1 O 2 O 2+ O 3

See attached sheets

Unknown

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES O 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		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
1) Stbl3 competent E.coli	Virapower lentiviral vector	InVitrogen	Fluc/mrfp/HK reporters	expression of reporter genes
2) TOP10	pBS (obtained from Cyp Fang)	InVitrogen	"	"

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Lentivirus	see 4.2	see 4.2	see 4.2	see 4.2

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify see attached sheets O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

4.6 Will virus be infectious to humans or animals? O YES O NO

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used mdx ; mdx:utrn-/- mice

6.3 AUS protocol # 2010-067

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

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

_____

_____

See E-mail

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Will live animals be used? O YES O No

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

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

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

_____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) CTX cardiotoxin (see attached)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin Mice 1.5mg/Kg iv

8.4 How much of the toxin is handled at one time*? 50µl of 10µM stock

8.5 How much of the toxin is stored*? 1mg

8.6 Will any biological toxins be used in live animals? ~~O~~YES, Please provide details: _____ O NO
CTX is well-reported for use to locally damage muscle, aiding in engraftment of transplanted cells

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

9.0 Insects

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____

_____

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

_____

_____

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

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

_____

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

_____

_____

_____

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

_____

_____

11.0 Import Requirements

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

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

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

11.4 Has the import permit been sent to OHS? O✓YES, please provide permit # P-13048 O NO
11-2007-00178-4

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE Terry Mr Lee
Lisa Hotte

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus Bio - LHR1 -0048
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

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

_____
_____

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

SIGNATURE Tony Wu Lee Date: Jan 18 2014
Tony Wu Lee January 14, 2014

15.0 Approvals

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

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

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

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

Special Conditions of Approval:

Subject: Re: Biological Agents Registry Form (Lee & Hoffman)
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Wed, 19 Jan 2011 17:46:06 -0500
To: Jeff Tucker <Jeff.Tucker@sjhc.london.on.ca>

HI Jeff -
Please see note #2 regarding the PHAC permit for the Dhavantari lab.
Regards,
Jennifer

On 1/18/2011 1:57 PM, Jennifer Stanley wrote:

> Hi Dr. Lee, Dr. Hoffman & Jennifer -
>
> Thanss for the Biological Agents Registry Form - I received the form in the mail today.
>
> I believe that this is for permit BIO-LHRI-0083, not permit BIO-LHRI-0048 (an expired Dhanvantari permit).
>
> I have a couple of questions:
>
> 1. In Section 6.0 (question 6.4) , is it cardiotoxin that is used in AUS protocol 2010-067?
>
> 2. Please confirm that Dr. Dhavantari has informed PHAC that your lab has possession of the "ViraPower Promoterless Lentiviral Gateway System" - this is requirement #6 under P-13043.
>
> Regards,
> Jennifer

Designations:	LoVo
Depositors:	M Romsdahl
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** colon **Tumor Stage:** Dukes' type C, grade IV **Disease:** colorectal adenocarcinoma **Derived from metastatic site:** left supraclavicular region
Cellular Products:	carcinoembryonic antigen (CEA) 908 ng/10 exp6 cells/10 days
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
Oncogene:	myc +; myb + ; ras +; fos +; p53 +; sis -; abl -; ros -; src -
Antigen Expression:	HLA A11, B15, B17, Cw1, Cw3; blood type B
DNA Profile (STR):	Amelogenin: XY CSF1PO: 10,11,13,14 D13S317: 8, 11 D16S539: 9, 12 D5S818: 11, 12, 13 D7S820: 9,3,10, 11 TH01: 9.3 TPOX: 8,9 vWA: 17,18
Cytogenetic Analysis:	The stemline chromosome number is hyperdiploid with the 2S component occurring at about 2.7% and 3 marker chromosomes were common to all S metaphases. Karyotypes were generally homogeneous and stable.
Isoenzymes:	ES-D, 1 G6PD, B PGD, A PGM1, 2 PGM3, 1-2
Age:	56 years
Gender:	male
Comments:	LoVo was initiated in 1971 from a fragment of a metastatic tumor nodule in the left supraclavicular region of a 56-year-old Caucasian male patient with a histologically proven diagnosis of adenocarcinoma of the colon. [1049] The cells are negative for expression of CSAp (CSAp-) and colon antigen 3. The line is positive for expression of c-myc, K-ras, H-ras, N-ras, Myb, sis and fos oncogenes. [22861] Myb, and fos oncogenes. [22861] N-myc and sis oncogene expression were not detected. [22861] Tumor specific nuclear matrix proteins CC-3 and CC-4 are expressed. [23341]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:10 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until

Related Links

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Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

Cell line info

Designations:	PC-3
Depositors:	ME Kaighn
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent (The cells form clusters in soft agar and can be adapted to suspension growth)
Organism:	*Homo sapiens* (human)
Morphology:	epithelial

Source:	**Organ:** prostate **Tumor Stage:** grade IV **Disease:** adenocarcinoma **Derived from metastatic site:** bone
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
Antigen Expression:	HLA A1, A9
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 11 D16S539: 11 D5S818: 13 D7S820: 8,11 THO1: 6,7 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	The line is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.
Age:	62 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. [22363] The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

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Cell Micrograph

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Related Cell Culture Products

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Product Information Sheet

Designations:	NCI-H1299
Depositors:	AF Gazdar; JD Minna
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** lung **Disease:** carcinoma; non-small cell lung cancer **Derived from metastatic site:** lymph node
Cellular Products:	neuromedin B
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 line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor 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 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 these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056; FAX (214) 688-7233.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin:X CSF1PO:12 D13S317:12 D16S539:12,13 D5S818:11 D7S820:10 THO1:6,9.3 TPOX:8 vWA: 16,17,18
Age:	43 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells have a homozygous partial deletion of the p53 protein, and lack expression of p53 protein. They reported to be able to synthesize the peptide neuromedin B (NMB) at 0.1 pmol/mg protein, but not the gastrin releasing peptide (GRP).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.

Related Links

NCBI Entrez Search

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

Designations:	HT-29
Depositors:	J Fogh
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial

Source:	**Organ:** colon **Disease:** colorectal adenocarcinoma
Cellular Products:	secretory component of IgA; carcinoembryonic antigen (CEA); transforming growth factor beta binding protein; mucin
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:** 1964
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	human adrenergic alpha2A [23560] urokinase receptor (u-PAR) vitamin D (moderate expression) urokinase receptor (u-PAR); vitamin D (moderate expression)
Tumorigenic:	Yes
Oncogene:	myc +; ras +; myb +; fos +; sis +; p53 +; abl -; ros -; src -
Antigen Expression:	Blood Type A, Rh+; HLA A1, A3, B12, B17, Cw5
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 11,12 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 6,9 TPOX: 8,9 vWA: 17,19
Cytogenetic Analysis:	modal number = 71; range = 68 to 72. The stemline chromosome number is hypertriploid with the 2S component occurring at 24%. Seventeen marker chromosomes are found in most metaphases, generally in single copy per chromosome. The marker designations are: M1p-(=t(3p-?) with a deleted short arm), t(7q;?), t(10q;?), i(13q), 19q+a, M6, ?t(8q;9q-), ?Xp, M9, 6q+, t(13;?)a, t(13;?)b, 18q+b, M14, M15, 15p+, and Xq-. Chromosome 13 is nullisomic and chromosomes 8 and 14 are generally monosomic. No Y chromosome was detected by QM band analysis.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1-2 Me-2, 1 PGM1, 1-2 PGM3, 1-2

Related Links

NCBI Entrez Search

Cell Micrograph

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Related Cell Culture Products

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Product Information Sheet

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial

Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+ and a single Xp+ in most cells.
Age:	fetus
Comments:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406] The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Related Links

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Product Information Sheet

Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences.]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney
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 line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell, Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
DNA Profile (STR):	Amelogenin: X CSF1PO: 11, 12 D13S317: 12, 14 D16S539: 9, 13 D5S818: 8, 9 D7S820: 11 THO1: 7, 9.3 TPOX: 11 vWA: 16, 18, 19
Age:	fetus
Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus. 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability 293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping, do not agitate the cells by hitting or

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Product Information Sheet

Cell Biology

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

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

Related Links

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Cell Micrograph

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Related Cell Culture Products

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Product Information Sheet

BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

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

BioStandards

Biological Reference Material and Consensus Standards for the life science community

Designations:	C6
Depositors:	G Sato
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	fibroblast
Source:	**Organ:** brain **Disease:** glioma **Cell Type:** glial cell
Cellular Products:	S-100 protein; produce glyceryl phosphate dehydrogenase in response to glucocorticoids; somatotrophin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	glucocorticoid
Virus Resistance:	poliovirus 3
Cytogenetic Analysis:	Stemline number is diploid. Karyotype is stable within the stemline number and is that of a normal male. Three cells with breaks; one with a secondary constriction, one with a dicentric, one with a rearrangement and four with terminal or centromere associations.
Comments:	The glial cell strain, C6, was cloned from a rat glial tumor induced by N-nitrosomethylurea by Benda et al. after a series of alternate culture and animal passages [PubMed: 4873531]. S-100 production increases ten fold as cells grow from low density to confluency.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 2.5%; horse serum to a final concentration of 15%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended. **Medium Renewal:** 2 to 3 times per week.
Preservation:	**Freeze medium:** culture medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020 recommended serum:ATCC 30-2040 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank's BSS (w/o Ca++

Related Links

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Related Cell Culture Products

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Product Information Sheet

Designations:	H9c2(2-1)
Depositors:	W Carlisle
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	myoblast
Source:	**Strain:** BD1X **Organ:** heart **Tissue:** myocardium
Cellular Products:	myokinase; creatine phosphokinase; myosin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Receptors:	acetylcholine; expressed
Age:	embryo
Comments:	H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue by B. Kimes and B. Brandt and exhibits many of the properties of skeletal muscle. Myoblastic cells in this line will fuse to form multinucleated myotubes and respond to acetylcholine stimulation. Fusion occurs faster if the serum concentration in the medium is reduced to one percent.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** The myoblastic population will become depleted rapidly if the cultures are allowed to become confluent. To prevent loss of myoblastic cells, cultures should be subcultured before they become confluent, and the line should be recloned periodically with selection for myoblastic cells. 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended. **Medium Renewal:** Every 2 to 3 days.
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO. **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	1062: Kimes BW, Brandt BL. Properties of a clonal muscle cell line

Related Links

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Product Information Sheet

– MSDS –

MATERIAL SAFETY
DATA SHEET

Note: this product is distributed in so minor quantities that, despite a possibly high level of toxicity, its potential hazard on the environment is negligible.

Cat. No. L81 02 December 6, 1994 Revised J.T. St. Joseph's Aug 31, 2009

CARDIOTOXIN

SECTION I IDENTIFICATION

Chemical Name: CARDIOTOXIN
Synonym: CYTOTOXIN
Formula: 60-amino acid peptide with 4 S-S bridges.

SECTION II HAZARDOUS INGREDIENTS DATA

Hazardous Components: Same as section I (single compound)

SECTION III PHYSICAL DATA

Boiling Point	:	N/A	**Volatile by Volume**	:	N/A
Vapor Pressure at Temperature	:	N/A	**Evaporation Rate (Butyl Acetate = 1)**	:	N/A
Vapor Density	:	N/A	**Specific Gravity**	:	N/E
Solubility in water	:	Good	**Melting Point**	:	N/A
Appearance and odor	:	Crystalline or amorphous powder.			

SECTION IV EXPLOSION AND FIRE HAZARD DATA

Flash Point: : N/A **Flammable Limit:** N/A
Test Mode : —

Extinguishing Media: Water, carbon dioxide, dry chemical powder, foam.
Special Fire Fighting Procedure: Firefighters must wear self-contained breathing apparatus and fully protective equipment.

Unusual Fire and Explosion Hazards: Smoke or fumes from burning may be toxic or irritating.

N/A = Not Applicable N/E = Not Established

SECTION V HEALTH HAZARD

Biological activity: Protein Kinase C inhibitor.

Toxicity: LD_{50} (mice, i.v.): 1.5 mg/kg
Route(s) of Entry: **Inhalation:** N/E **Skin:** N/A **Ingestion:** Yes
Health Hazards (acute and chronic): Toxic.
Medical Conditions Generally Aggraved by exposure: N/E

Signs and Symptoms of Exposure: N/E.

Emergency and First Aid Procedures:

Skin contact	:	Wash affected area with copious amounts of water.
Eye Contact	:	Flush eyes with water for at least 15 minutes.
Inhalation	:	Remove to fresh air. Give oxygen or artificial respiration as needed.
Ingestion	:	Call physician immediately.

Seek medical treatment if discomfort persists.

SECTION VI REACTIVITY DATA

Stability	:	Stable.
Conditions to Avoid	:	N/E
Incompatibility	:	N/E
Hazardous Decomposition Products	:	N/E
Hazardous polymerisation	:	N/A

SECTION VII SPILL OR LEAK PROCEDURES

Steps to be Taken in case Material is Released or Spilled: Wear self-contained breathi apparatus, rubber boots and gloves. Sweep up. Do not raise dust. Wash and ventilate spill after pickup is complete. Do not allow material or wash water to enter natural waterway.

Waste Disposal Method: Mix waste with a combustible carrier and burn in a suitable equi chemical incinerator.

Disposal must comply with Federal, state and local regulations.

SECTION VIII SPECIAL PROTECTION INFORMATION

Respirator Protection	:	Mechanical filter type.
Ventilation	:	Mechanical.
Protective Gloves	:	Nitrile rubber (J.T. St. Joseph's Aug 31, 2009)
Eye Protection	:	Goggles.
Other Protective Equipment	:	Store dry in tight container.

Should be handled only by qualified, experienced professionals.

SECTION IX SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storage: Keep storage container tightly closed prolonged or frequent exposure. Wash thoroughly after handling.

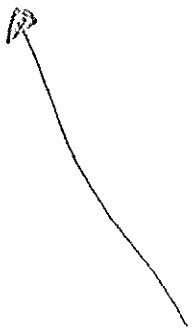
Toxin Info

Cardiotoxin will be used to damage the muscle to improve SC transplantation. This treatment will by itself increase the success of myoblast tramsplantation. If used in combination with irradiation for the initial transplantation, this will further improve the transplantation success. Cardiotoxin injection will be repeated 2-3 weeks after cell implantation, will further increase the success of transplantation, and will induce a proliferation of the SCs initially transplanted. We do not expect pain to be caused by the injection, there should just be localized irritation and minor muscle damage enough to help the cell transplant. However the mouse will be checked daily to be sure that it is not having any walking problems or unexpected outcomes from the injection.

------ Original Message ------
From: "Jennifer Stanley" <jstanle2@uwo.ca>
To: "Jennifer Hadway" <jhadway@lawsonimaging.ca>
Cc: "Lisa Hoffman" <lhoffman@lawsonimaging.ca>
Sent: Monday, September 14, 2009 5:05 PM
Subject: Re: Biohazard Modification Form: Lee (AUS 2008-067)]

Hi Jen
Can you tell me
1. LD50 for the toxin (and species) Mice 1.5mg/kg iv
2. How much of the toxin is handled at one time? 50ul of 10uM stock
3. How much of the toxin is stored? 1mg

Thanks
Jennifer



$1.5\ \frac{mg}{kg} \times \frac{50\ kg}{1\ person} = 75\ mg$

conservative
(small person)

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

Public Health Agency of Canada
Centre for Emergency Preparedness and Response

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

Permit to import human pathogen(s)

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

P-13043

Under the authority of the Human Pathogens Importation Regulations.

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

Importer-Name, address and postal code - Importateur-Nom, adresse et code postal	Facsimile-Télécopieur	Telephone no.- No. de téléphone
Lawson Health Research Institute 268 Grosvenor Street London, ON N6A 4V2 Attn.: Dr. Savita Dhanvantari	(519) 646-6110	(519) 646-6100 ext.: 65738

Supplier-Name and address - Fournisseur-Nom et adresse	Name(s) of Port(s) of Entry- To Clear Customs at Port(s) of entry Nom(s) de(s) point(s) d'entrée -Dédouanement au(x) point(s) d'entrée
Invitrogen Corporation Inc. 1600 Faraday Ave., Carlsbad, CA 92008, USA	Various ports

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

ViraPower Promoterless Lentiviral Gateway Expression System (cat# K5910-00)*.

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

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

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

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

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

■ b) importations at intervals of
les importations effectuées à intervalles de

during the period beginning on
au cours de la période commençant le

September 27, 2006

and ending on
et se terminant le

September 30, 2007

Authorization-Signature of Director
Autorisation-Signature du Directeur

Paul J. Payette, Ph.D.

Date September 27, 2006

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

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

Canada

Canadian Food Inspection Agency
Goverment of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2007-00178-4
ORIGINAL
2007/01/12
year/mo/day
année/mois/jour

IMPORT PERMIT PERMIS D'IMPORTATION

THIS PERMIT IS ISSUED PURSUANT TO:/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A.
THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur
LAWSON RESEARCH INSTITUTE

268 GROSVENOR STREET, ROOM H417
LONDON, ONTARIO
N6A4V2

Contact: Dr. Savita Dhanvantari Applicant Name: DR. SAVITA DHANVANTARI
Phone: (519) 646-6100 ext. 65738 Fax: (519) 646-6110

Exporter/Exportateur
INVITROGEN CORPORATION INC.

1600 FARADAY AVENUE
CARLSBAD CALIFORNIA
UNITED STATES
440190
Contact: Mike Galleno

Phone: (760) 603-7219 Fax: (760) 602-6519

Quarantine/Destination/Quarantaine

Producer/Producteur

Valid/Valide from/du 2007/01/12 year/month/day année/mois/jour to/au 2008/01/31 year/month/day année/mois/jour

Country of Origin/ Pays d'Origine UNITED STATES

For the entry of/ Pour l'entrée de: ____ Single shipment/Chargement simple XX Multiple shipments/Chargements multiples

Place of entry into Canada/Lieu d'entrée au Canada:
Various Ports of Entry

FOR THE IMPORTATION OF:/POUR L'IMPORTATION DE:
(Description of things(s)/Description de la ou des choses)
1. Product Description: ONE OR MORE OF THE INVITROGEN LENTIVIRAL PRODUCTS LISTED ON THE ATTACHMENT TITLED "ATTACHMENT TO ANIMAL PATHOGEN(S) IMPORT PERMIT # A-2007-00178-4.

(TO BE USED IN ROOM 4-508, CULTURE ROOM F4-127A, LAWSON HEALTH RESEARCH INSTITUTE, LONDON, ON.) Proposed End Use: "In Vitro" Scientific Name: Biocontainment Level: 2

A PERSON WHO IMPORTS A THING UNDER THIS PERMIT SHALL COMPLY WITH ALL THE CONDITIONS SET OUT HEREIN/TOUTE PERSONNE QUI IMPORTE UNE CHOSE EN VERTU DE CE PERMIS DEVRA RESPECTER TOUTES LES CONDITIONS DÉCRITES CI-DESSOUS

Selected Conditions / Conditions Choisies

ONE OR MORE OF THE INVITROGEN LENTIVIRAL PRODUCTS LISTED ON THE ATTACHMENT TITLED "ATTACHMENT TO ANIMAL PATHOGEN(S) IMPORT PERMIT # A-2007-00178-4.

(TO BE USED IN ROOM 4-508, CULTURE ROOM F4-127A, LAWSON HEALTH RESEARCH INSTITUTE, LONDON, ON.)

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

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

Canada

Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
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ORIGINAL
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IMPORT PERMIT PERMIS D'IMPORTATION

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

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

Importer/Importateur	Exporter/Exportateur
LAWSON RESEARCH INSTITUTE 268 GROSVENOR STREET, ROOM H417 LONDON, ONTARIO N6A4V2 Contact: Dr. Savita Dhanvantari Applicant Name: DR. SAVITA DHANVANTARI Phone: (519) 646-6100 ext. 65738 Fax: (519) 646-6110	INVITROGEN CORPORATION INC. 1600 FARADAY AVENUE CARLSBAD CALIFORNIA UNITED STATES 440190 Contact: Mike Galleno Phone: (760) 603-7219 Fax: (760) 602-6519

Selected Conditions / Conditions Choisies (Continued/Suite)

3. The imported material must be packaged in appropriate shipping containers to prevent accidental spillage of contents during shipping. Importers should be aware of their obligations under Transport Canada's regulations concerning transportation of dangerous goods.

4. All infectious material must be handled in appropriate animal pathogen containment level 2 facilities as described in Containment Standards for Veterinary Facilities, 1996, AAFC publication no. 1921.

5. The material authorized for importation by this permit is to be used in in vitro studies ONLY and must not to be introduced into laboratory, domestic or wild animals (including birds or fish) unless written authorization is obtained from the Canadian Food Inspection Agency.

6. The animal(s) or thing(s) imported under this permit must not be removed from the premises of destination listed on this permit, unless written authorization is obtained from the Canadian Food Inspection Agency.

7. Upon completion of the tests or experiments, the imported material as described on this permit and any derivatives thereof must be autoclaved, incinerated or alternatively disposed of in a manner approved by an inspector of the Canadian Food Inspection Agency.

8. Records pertaining to the imported product's use, storage and disposal must be maintained for two (2) years following importation. These records must be made available for inspection by the Canadian Food Inspection Agency upon request.

9. The importer is responsible for all costs incurred or associated with any testing or treatment of the animal(s) or thing(s) that may be required under the import permit or under the authority of the Health of Animals Act or the Health of Animals Regulations. The importer shall pay all fees for services required in respect of the importation under the National Animal Health Program Cost Recovery Fees Regulations in place at the time of importation.

10. Consideration of an application necessary for issuance of a permit to import the described animal or thing is subject to Class I fees.

11. The issuance of this permit does not relieve the owner or the importer of the obligation to comply with any other relevant federal, provincial or municipal legislation or requirement.

12. Failure to comply with the conditions contained in this permit or with the provisions of the Health of Animals Act and Regulations may result in the cancellation of this permit and will result in the forfeiture to the Crown of the imported thing(s) or in the removal of the thing(s) from Canada, all without compensation to, and at the expense of the importer. The importer(s) are responsible for the imported thing(s), their freedom from extraneous disease, active or latent, and genetic or other defects. The importer, his heirs, executors, successors and assigns release and discharges Her Majesty the Queen in right of Canada and the CFIA of and from all claims and demands, damages, actions or causes of action arising or to arise by reason of the importation of the thing(s) and agrees to indemnify and save harmless Her Majesty the Queen in right of Canada and the CFIA from and against all actions, damages, claims

Canadian Food Inspection Agency
Goverment of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2007-00178-4
ORIGINAL
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IMPORT PERMIT PERMIS D'IMPORTATION

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

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

Importer/Importateur	Exporter/Exportateur
AWSON RESEARCH INSTITUTE 68 GROSVENOR STREET, ROOM H417 ONDON, ONTARIO 6A4V2 ontact: Dr. Savita Dhanvantari Applicant Name. DR. SAVITA DHANVANTARI hone: (519) 646-6100 ext. 65738 Fax: (519) 646-6110	INVITROGEN CORPORATION INC. 1600 FARADAY AVENUE CARLSBAD CALIFORNIA UNITED STATES 440190 Contact: Mike Galleno Phone: (760) 603-7219 Fax: (760) 602-6519

Selected Conditions / Conditions Choisies (Continued/Suite)

ad demands which may be brought in respect of or arising out of the importation of such thing(s), any contamination with extraneous disease or other efects.

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

Additional Conditions Additionnelles

NE OR MORE OF THE INVITROGEN LENTIVIRAL PRODUCTS LISTED ON THE ATTACHMENT TITLED "ATTACHMENT TO ANIMAL ATHOGEN(S) IMPORT PERMIT # A-2007-00178-4.

TO BE USED IN ROOM 4-508, CULTURE ROOM F4-127A, LAWSON HEALTH RESEARCH INSTITUTE, LONDON, ON)

. No culturing of containment level 3 or 4 pathogens shall be done.

Authorized By:/Approuvé par:
CINTHIA LABRIE

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

Canada

Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Advice and Biohazards Division
Science Strategies Directorate, CFIA
159 Cleopatra Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Division des avis scientifiques et contrôle des biorisques
Direction des stratégies scientifiques, ACIA
159 promenade Cleopatra, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

ATTACHMENT TO ANIMAL PATHOGEN(S) IMPORTATION PERMIT
ATTACHEMENT AU PERMIS D'IMPORTATION D'AGENTS ZOOPATHOGÈNES
#A-2007-00178-4

Issued to/ Délivré à: Dr. Savita Dhanvantari, Lawson Health Research Institute,
268 Grosvenor Street, London ON N6A 4V2.

Includes the following animal pathogen containment Level 2 microorganisms:
Inclut les agents zoopathogènes de niveau de confinement 2 suivant:

Invitrogen Lentiviral Products / Produits Lentiviral d'Invitrogen:

- PCDNA6.2/C-EMGFP-GW/TOPO (K35920)
- PCDNA6.2/N-EMGFP-GW/TOPO (K36020)
- PCDNA6.2/C-YFP-GW/TOPO (K36120)
- PCDNA6.2/N-YFP-GW/TOPO (K36620)
- Virapower II Lenti GW System (K36720)
- Virapower II Lenti C-Lumio system (K37020)
- Virapower II Lenti N-Lumio system (K37120)
- POL III MIR Rnai Vector (K493500)
- POL II MIR Rnai GFP Vector (K493600)
- Lenti POL II MIRE Rnai Vector (K493700)
- Lenti POL II MIRE Rnai w/GFP (K493800)
- Block it Lenti RNAi Expression system (K494400)
- Virapower Lentiviral directional (K495000)
- Virapower Lentviral Gateway (K496000)
- Lentiviral T Rex Expression system (K496500)
- Virapower packaging mix (K497500)
- Virapower Zeo Lenti Expression (K498000)
- Virapower Zeo Lentiviral Support Kit (K498500)
- Virapower UBC Lenti expression (K499000)
- Virapower Lentiviral support (K497000)
- Plenti6/Block it RNAi vector (K494300)
- Plenti 6/V5 Directional TOPO (K495510)
- VP TR GW Vector kit (K496700)
- PCDNA6.2/EMGFP-BSD/V5 Dest (V36620)
- Plenti6.2/V5-DEST GW vector (V36820)
- Plenti6.2-GW/EMGFP Exp vector (V36920)
- Plenti6/TR vector (V48020)
- Block-iT Lenti RNAi ZW GW Vector (V48820)
- Plenti6/V5 Gtwy vector pack (V49610)
- Plenti4/V5 -Dest Gateway vector (V49810)
- Plenti6/UBC/V5 Dest vector (V49910)
- Block it Lentiviral Inducible RNAi (K492500)
- Promotorless Lenti Exp kit (K591000)

The above products may contain one or more of the following components / Les produits ci-dessus peuvent contenir un ou plusiers des composants suivants:

Plenti6/Block it Dest RNAi , PLP1, PLP2, PLP3/VSVG, Plenti6/V5-Dtopo, Plenti6/V5-GW/LacZ, plenti6/V5 Dest vector, plenti6/TR, plenti4/TO/V5 Dest, plenti4/TO/V5-GW/LacZ, plenti 4/V5 Dest, plenti4/V5 -GW/LacZ vector, plenti4/Blockit Dest, plenti6/UBC/ V5 Dest vector, plenti6/UBC/V5-GW/LacZ vector, plenti6/R4R2/V5-Dest, 293 FT cells, PCDNA6.2-GW/MIR Neg TB, PCDNA6.2-GW-EMGFP-MIR Neg, Plenti6.2/C-Lumio/V5 DEST,Plenti 6.2/C-Lumio-V5-GW/LA, Plenti6.2/N-Lumio/V5 Dest, Plenti6.2/N-Lumio/V5-GW/LA, Plenti6.2-GW/EMGFP Kit, Plenti 6.2.V5 Dest Kit, Plenti6.2/V5-GW LacZ, PCDNA6.2/EMGFP-BSD/V5 Dest, PCDNA6.2/EMGFP-BSD/V5-GW/C, PCDNA6.2/C-EMGFP-GW, PCDNA6.2/C-EMGFP-GW/CAT, PCDNA6.2/N-EMGFP-GW, PCDNA6.2/N-EMGFP-GW, PCDNA6.2/C-YFP-GW, PCDNA6.2 C-YFP-GW/CAT, PCDNA6.2/N-YFP-GW, PCDNA6.2/N-YFP-GW-CAT.

REVISED: May 01, 2006.

Cinthia Labrie
Cinthia Labrie
A/Chief, Animal Pathogen Importation Program/
Chef intérimaire, Programme d'importation des agents zoopathogènes

jan 12/07
Date

Canada

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

❊ IMPORTANT NOTICE ❊

Your file / Votre référence

Our file / Notre référence

1) ZOONOTIC IMPORTS: Please check the **"Description of Pathogen(s)"** section of your attached permit, and **if** the following message (in red print) has been included: " ***Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for importation.**", then the material is of a **zoonotic** nature and a valid permit from the Canadian Food Inspection Agency (CFIA) is required for this importation in addition to your attached human pathogens import permit. If you do not have a valid permit from the Canadian Food Inspectin Agency, please contact them directly for assistance at: **(613) 221-7068.**

2) INSTRUCTIONS FOR USE OF YOUR PERMIT:
[as per the *Human Pathogen(s) Importation Regulations (SOR/94-558)*]

Prior to shipment of the human pathogen described in the Import Permit the importer **must**:

a) provide a copy of the importation permit to the supplier and notify the supplier that **a copy of the importation permit must be attached to each shipment**;

b) **notify the supplier** that the outer shipping container in which the human pathogen is transported must display clearly, on the outside surface of the container, the importation permit number and the following statement immediately preceeding that number:

"Human Pathogen – Importation Permit Number:/Agent anthropopathogène – Numéro du permis d'importation:"

If the permit holder who arranges to import a human pathogen that belongs to Risk Group 3 or 4, does not receive the human pathogen on, or within three (3) days after, such date of receipt as may reasonably be expected in the circumstances, he shall forthwith give to the Director, Office of Laboratory Security a notice that the human pathogen has not been received and provide the Director with the importation permit number.

To facilitate Customs clearance, a copy of the importation permit should be kept by the importer and presented to Customs or sent to the importer's customs broker.

3) Please note that importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca for assistance.

Direct inquiries to:
Office of Laboratory Security
Public Health Agency Canada
Centre for Emergency Preparedness and Response
100 Colonnade Road, Loc.: 6201A
Ottawa, Ontario K1A 0K9

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

Canada

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

Office of Biohazard Containment and Safety
Science Advice and Biohazards Division
Science Strategies Directorate, CFIA
159 Cleopatra Drive, Ottawa, Ontario K1A 0Y9

Bureau du confinement des biorisques et sécurité
Division des avis scientifiques et contrôle des biorisques
Direction des stratégies scientifiques, ACIA
159 promenade Cleopatra, Ottawa, Ontario K1A 0Y9

FACSIMILE TRANSMITTAL NOTICE / TRANSMISSION PAR TÉLÉCOPIEUR

To / À:	*From / De:*
Dr. Sativa Dhanvantari Lawson Research Institute	Andrew Halliday Animal Pathogen Import Program / Programme d'importation des agents zoopathogènes
Facsimile/télécopieur: 519-646-6110	*Facsimile/télécopieur:* 613-228-6129

Subject/Objet: **Importation of animal pathogens / Importation d'agents zoopathogènes**

Message:

Please find attached / Veuillez trouver ci-joint :

☐ A copy of a Non-pathogenic letter for the product(s) you requested./ Une copie de la lettre de non-pathogénéicité pour le(s) produit(s) demandé(s).

☐ A copy of the import permit for which you applied. Please review the conditions appearing on your permit. / Une copie de votre permis d'importation. Veuillez s'il-vous-plaît prendre note des conditions apparaissant sur votre permis.

☐ Condition # 13: The product(s) requested is(are) also regulated by the Public Health Agency of Canada (PHAC). Please contact PHAC at (613) 957-1779. / Le(s) produit(s) demandé(s) sont également réglementés par l'Agence de santé publique du Canada (ASPC). Veuillez contacter l'ASPC au (613)-957-1779.

Andrew Halliday
importzoopath@inspection.gc.ca

Please visit our website at: http://www.inspection.gc.ca/english/sci/bio/bioe.shtml.
Veuillez visiter notre site internet au: http://www.inspection.gc.ca/francais/sci/bio/biof.shtml.

Signature:	*Date:*	*Telephone/Téléphone:*	*No./Nbre Pages:*
	January 12, 2007	613-221-7068	5

invitrogen

QUOTATION

IN RESPONSE TO YOUR INQUIRY

TO ORDER:
Invitrogen Canada Inc
2270 Industrial Street,Burlington,ON L7P 1A1
To Order (800) 263-6236
Fax No (800) 387-1007
E-mail caorders@invitrogen.com

TO

LAWSON RESEARCH INSTITUTE
FOR: Dr. Dhanvantari/Lisa Hoffman

LONDON
ON N6A 4V2 Canada
ATTN: Lisa Hoffman

QUOTATION NO.: S6912311 _ 8

To ensure correct pricing and terms the above quote number must appear on all orders and correspondence

FROM: 07/21/2006 THROUGH 07/20/2007

EXCEPT WHERE NOTED BELOW

TERMS: NET 30 DAYS

ESTIMATED DELIVERY DAYS, A.R.O.

FOB: Shipping Point

To place an order please call Customer Service
1-800-263-6236

WE ARE PLEASED TO QUOTE ON YOUR REQUIREMENTS AS FOLLOWS:
NOUS AVONS LE PLAISIR DE VOUS ENVOYER LA SOUMISSION CORRESPONDANT À VOTRE REQUÊTE :

Natalie Shier — Territory Sales Manager 861

ITEM NO	CATALOG NO	DESCRIPTION	QUALIFYING LIMIT	PRICE OR % DISCOUNT	
				DISCOUNT/UNIT	EXTENDED/UNIT
1	K591000	ViraPower™ Promoterless Lentiviral Gateway® Expression System with MultiSite™ Gateway® Technology 1 kit	1+	$1,575.00	$1,575.00

TERMS AND CONDITIONS

Telephone Number (Continue)

THESE GOODS ARE FOR RESEARCH ONLY,UNLESS OTHERWISE SPECIFIED SEE "AUTHORISED USERS" IN GENERAL TERMS AND CONDITIONS
A moins d'indications contraires, ces produits sont destinés à la recherche. Voir la section "Utilisations autorisées" dans les conditions générales

invitrogen

QUOTATION

IN RESPONSE TO YOUR INQUIRY

TO ORDER
Invitrogen Canada inc
2270 Industrial Street,Burlington,ON L7P 1A1
To Order (800) 263-6236
Fax No (800) 387-1007
E-mail caorders@invitrogen.com

TO

LAWSON RESEARCH INSTITUTE
FOR Dr. Dhanvantari/Lisa Hoffman

LONDON
ON N6A 4V2 Canada
ATTN Lisa Hoffman

QUOTATION NO.: S6912311 _ B

To ensure correct pricing and terms, the above quote number must appear on all orders and correspondence.

FROM 07/21/2006 THROUGH 07/20/2007

EXCEPT WHERE NOTED BELOW

TERMS: NET 30 DAYS

ESTIMATED DELIVERY DAYS A.R.O

FOB: Shipping Point

To place an order please call Customer Service
1-800-263-6236

WE ARE PLEASED TO QUOTE ON YOUR REQUIREMENTS AS FOLLOWS:
NOUS AVONS LE PLAISIR DE VOUS ENVOYER LA SOUMISSION CORRESPONDANT À VOTRE REQUÊTE :

TERMS AND CONDITIONS

ADDITIONAL TERMS AND CONDITIONS OF QUOTATION

1 General Terms and Conditions listed on the customer copy of packing lists and invoices from Invitrogen Corporation will apply except where otherwise agreed in writing by an authorized representative of Invitrogen Corporation.

2 In order to receive quoted prices, the quotation number must be referenced at time of order. Credits will not be issued for orders not referencing quotation numbers

3 The effective dates of this quotation appear in the upper right corner of each page unless otherwise noted. Exceptions are noted with n the body of this quotation

4. The quantities noted on this quotation reflect minimum order requirements necessary to receive quoted prices.

5 Percentage discounts will be calculated from current list price.

6. This quotation may be terminated by Invitrogen Corporation upon written notice.

7 This quotation contains confidential Invitrogen Corporation pricing information which if disclosed to third parties could cause competitive harm to Invitrogen Corporation. Subject to overriding obligations to third party funding agencies or governmental entities, the customer agrees to keep all pricing information contained herein confidential ..

IF OUR SUPPLIER COSTS CHANGE DURING THE DURATION OF THIS QUOTE, YOUR PRICES MAY BE ADJUSTED

Telephone Number (Continue)

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

Date: September 27, 2006

Your file / Votre référence

Our file / Notre référence

Importer address: Lawson Health Research Institute
268 Grosvenor St.
London, ON
N6A 4V2

Dear Dr. Savita Dhanvantari,

Enclosed you will find your Public Health Agency of Canada permit to import human pathogen(s), **P-13043.**

Due to the nature of the material requested for import, some additional conditions apply. Please review and note the conditions of import, in particular conditions #8, #9 and #10. Condition #8 states that "Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements. No culturing of Risk Group 3 pathogens shall be done". Condition #9 states that "No imported material may be removed to another location, or transferred into the possession of a person other than the importer, without the permission of the director [of the Office of Laboratory Security, Public Health Agency of Canada]". Condition #10 states that "The Director [of the Office of Laboratory Security, Public Health Agency of Canada] must approve all new work with the imported material involving construction of recombinants that require an increase of containment from level 2".

If you have any questions or comments regarding this matter, please do not hesitate to contact our office.

Sincerely,

Paul J. Payette, Ph.D.
Director, Office of Laboratory Security
Centre for Emergency Preparedness and Response
100 Colonnade Road, Loc.: 6201A
Ottawa, Ontario, Canada K1A 0K9
Phone: (613) 957-1779
Fax: (613) 941-0596

Encl.

Canada

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

❊ IMPORTANT NOTICE ❊

Your file *Votre référence*

Our file *Notre référence*

1) ZOONOTIC IMPORTS: Please check the **"Description of Pathogen(s)"** section of your attached permit, and **if** the following message (in red print) has been included: " ***Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for importation.**", then the material is of a **zoonotic** nature and a valid permit from the Canadian Food Inspection Agency (CFIA) is required for this importation in addition to your attached human pathogens import permit. If you do not have a valid permit from the Canadian Food Inspectin Agency, please contact them directly for assistance at: **(613) 221-~~7068~~ 7088** ANDREW.

2) INSTRUCTIONS FOR USE OF YOUR PERMIT:
[as per the *Human Pathogen(s) Importation Regulations (SOR/94-558)*]

Prior to shipment of the human pathogen described in the Import Permit the importer **must**:

a) provide a copy of the importation permit to the supplier and notify the supplier that **a copy of the importation permit must be attached to each shipment**;

b) **notify the supplier** that the outer shipping container in which the human pathogen is transported must display clearly, on the outside surface of the container, the importation permit number and the following statement immediately preceeding that number:

"Human Pathogen – Importation Permit Number:/Agent anthropopathogène – Numéro du permis d'importation:"

If the permit holder who arranges to import a human pathogen that belongs to Risk Group 3 or 4, does not receive the human pathogen on, or within three (3) days after, such date of receipt as may reasonably be expected in the circumstances, he shall forthwith give to the Director, Office of Laboratory Security a notice that the human pathogen has not been received and provide the Director with the importation permit number.

To facilitate Customs clearance, a copy of the importation permit should be kept by the importer and presented to Customs or sent to the importer's customs broker.

3) Please note that importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca for assistance.

Direct inquiries to:
Office of Laboratory Security
Public Health Agency Canada
Centre for Emergency Preparedness and Response
100 Colonnade Road, Loc.: 6201A
Ottawa, Ontario K1A 0K9

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

Canada

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

Your file / Votre référence

Our file / Notre référence

Dear Sir/Madam:

Please find attached, for your convenience and future use, an application form for a Public Health Agency of Canada permit to import human pathogens. When filling out this form, please note the following directives:

- If ordering from a commercial supplier (e.g. ATCC), please provide the product name, catalogue number and any relevant descriptive information. If the product is coming from another researcher, please provide background information (references, etc.).

- If your work objectives (Box 10) include *in vivo* activities, please describe in full, including animal species used.

When completed, please forward the **original application** to our office at the following address:

Office of Laboratory Security
Public Health Agency of Canada
Centre for Emergency Preparedness and Response
100 Colonnade Road, Loc.: 6201A
Ottawa, Ontario
K1A 0K9

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

Upon receipt of your application, a permit will be issued and faxed back to you (usually within 5 working days) and the original will follow through regular mail.

If you have any questions regarding this matter, please do not hesitate to contact our office.

Thank you for your collaboration.

Monsieur, Madame,

Vous trouverez sous pli, pour utilisation future, un formulaire de demande de permis de l'Agence de santé publique du Canada pour l'importation d'agent(s) anthropopathogène(s) . Nous vous prions de tenir compte des directives suivantes lorsque vous complétez le formulaire :

- Si vous commandez d'un fournisseur commercial (p.ex. l'ATCC), prière de nous fournir le nom du produit, le numéro de catalogue et toute l'information et/ou description qui s'y rattache. Si le produit doit vous parvenir d'un autre chercheur, prière de nous fournir toute l'information pertinente (références, etc.).

- Si l'objectif de votre travail (Section 10) comprend des activités *in vivo*, prière de nous fournir une description complète, incluant les espèces d'animaux utilisés.

Une fois complété, veuillez nous faire parvenir la **copie originale du formulaire de demande de permis** à l'adresse suivante :

Bureau de la sécurité des laboratoires
Agence de santé publique du Canada
Centre de mesures et d'interventions d'urgence
100 chemin Colonnade, Loc.: 6201A
Ottawa, Ontario
K1A 0K9

Tél.: (613) 957-1779
Fax: (613) 941-0596

Nous pouvons émettre un permis et vous le faire parvenir par télécopieur, habituellement dans les cinq jours suivant la réception du formulaire de demande. La copie originale du permis vous parviendra ensuite par la poste.

Pour de plus amples renseignements, n'hésitez pas à entrer en contact avec notre bureau.

Merci de votre collaboration.

Canada

InVitrogen

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Catalog no.
293FT Cell Line	R700-07
BioModule™ Lentiviral 293 Unit	WFGE08-S

Kit Components

The 293FT Cell Line and BioModule™ Lentiviral 293 Unit include the following components. For detailed contents, see the following pages.

The 293FT Cell Line and BioModule™ Lentiviral 293 Unit are shipped as described below. Upon receipt, store each item as detailed below.

Component	Catalog no.		Shipping	Storage
	R700-07	WFGE08-S		
293FT Cell Line	√		Dry ice	Liquid nitrogen
Dulbecco's Modified Eagle Medium (D-MEM)		√	Room Temperature	2°C to 8°C
10 mM MEM Non-Essential Amino Acids Solution (100X)		√	Room Temperature	2°C to 8°C
MEM Sodium Pyruvate Solution (100X)		√	Room Temperature	2°C to 8°C
Phosphate-Buffered Saline, pH 7.4		√	Room Temperature	2°C to 8°C
Opti-MEM® I Reduced Serum Medium		√	Room Temperature	2°C to 8°C (keep in the dark)
Geneticin® Selective Antibiotic (50 mg/ml)		√	Room Temperature	-20°C or 2°C to 8°C
Trypan Blue Stain		√	Room Temperature	Room Temperature
Fetal Bovine Serum		√	Dry ice	-5° to -20°C
200 mM L-Glutamine (100X)		√	Dry ice	-5° to -20°C
Penicillin-Streptomycin		√	Dry ice	-5° to -20°C
Trypsin-EDTA		√	Dry ice	-5° to -20°C

Continued on next page

Kit Contents and Storage, continued

293FT Cell Line

The 293FT Cell Line is used for the production of lentiviral stocks. The 293FT Cell Line is supplied as one vial containing 3 x 10^6 frozen cells in 1 ml of Freezing Medium. **Upon receipt, store in liquid nitrogen until use.**

Handle as potentially biohazardous material under at least Biosafety Level 2 containment. This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

BioModule™ Lentiviral 293 Unit

The following reagents are provided with the BioModule™ Lentiviral 293 Unit:

Component	Composition	Quantity
Dulbecco's Modified Eagle Medium	D-MEM high glucose (1X), containing 4,500 mg/L D-glucose, and 4 mM L-glutamine, but no sodium pyruvate.	2 x 1000 ml
10 mM MEM Non-Essential Amino Acids Solution (100X)	890 mg/L L-Alanine 1320 mg/L L-Asparagine 1330 mg/L L-Aspartic Acid 1470 mg/L L-Glutamic Acid 750 mg/L Glycine 1150 mg/L L-Proline 1050 mg/L L-Serine	100 ml
MEM Sodium Pyruvate Solution (100X)	100 mM Sodium Pyruvate Solution (11,004 mg/L)	100 ml
Phosphate-Buffered Saline, pH 7.4	0.144 g/L KH_2PO_4 9.00 g/L NaCl 0.795 g/L Na_2HPO_4 pH 7.4	500 ml
Opti-MEM® I Reduced Serum Medium	See below	500 ml
Geneticin® Selective Antibiotic (50 mg/ml)	50 mg/ml active Geneticin® Selective Antibiotic in distilled water	20 ml
Trypan Blue Stain	0.4% Trypan Blue solution in 0.85% NaCl	100 ml
Fetal Bovine Serum	Fetal Bovine Serum, Certified (US)	2 x 100 ml
200 mM L-Glutamine (100X)	200 mM L-Glutamine (29.2 mg/ml) in 0.85% NaCl	100 ml
Penicillin-Streptomycin	5,000 units/ml penicillin (base) 5,000 µg/ml streptomycin (base) in 0.85% NaCl	100 ml
Trypsin-EDTA	0.5 g/L trypsin (1:250) 0.2 g/L EDTA•4Na in Hanks' Balanced Salt Solution without $CaCl_2$, $MgCl_2 \cdot 6H_2O$, and $MgSO_4 \cdot 7H_2O$ Contains phenol red	100 ml

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Overview

Introduction

This manual is provided with the 293FT Cell Line and BioModule™ Lentiviral 293 Unit. The 293FT Cell Line is a very suitable host for lentiviral production, while the BioModule™ Lentiviral 293 Unit contains the reagents for optimal growth and lentiviral production of the 293FT Cell Line. Below the characteristics of the 293FT Cell Line and BioModule™ Lentiviral 293 Unit are explained.

293FT Cell Line

The 293FT Cell Line is derived from the 293F Cell Line (see below) and stably expresses the SV40 large T antigen from the pCMVSPORT6TAg.neo plasmid. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter and is high-level and constitutive. For more information about pCMVSPORT6TAg.neo, see the **Appendix**, page 10.

Use of the Cell Line

Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naldini *et al.*, 1996), making the 293FT Cell Line a particularly suitable host for generating lentiviral constructs using the ViraPower™ Lentiviral Expression System available from Invitrogen (Catalog nos. K4950-00 and K4960-00).

Parental Cell Lines

The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham *et al.*, 1977; Harrison *et al.*, 1977). The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein.

The 293-F Cell Line available from Invitrogen (Catalog no. 11625) is a fast-growing variant of the 293 cell line, and was originally obtained from Robert Horlick at Pharmacopeia.

Antibiotic Resistance

293FT cells stably express the neomycin resistance gene from pCMVSPORT6TAg.neo and should be maintained in medium containing Geneticin® at the concentration listed below. Expression of the neomycin resistance gene in 293FT cells is controlled by the SV40 enhancer/promoter.

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Introduction

Overview

Introduction

The ViraPower™ Promoterless Lentiviral Gateway® Expression System combines Invitrogen's ViraPower™ Lentiviral and MultiSite Gateway® technologies to facilitate lentiviral-based expression of a gene of interest from any promoter of choice in dividing or non-dividing mammalian cells. The System includes:

- The pENTR™5'-TOPO® TA Cloning Kit for production of an entry clone containing your eukaryotic promoter of interest. The pENTR™5'-TOPO® entry vector is adapted with MultiSite Gateway® Technology to facilitate transfer of the promoter sequence into the lentiviral expression plasmid.
- A promoterless pLenti6/R4R2/V5-DEST destination vector into which the promoter and gene of interest are transferred. This expression plasmid contains elements that allow packaging of the construct into virions and the Blasticidin resistance marker for selection of stably transduced cell lines.
- Components of the ViraPower™ Lentiviral System (Catalog no. K5910-00 only) for production of a replication-incompetent lentivirus that transiently or stably expresses the gene of interest in both dividing and non-dividing mammalian cells.

For more information about the ViraPower™ Lentiviral Technology and the MultiSite Gateway® Technology, see pages 6-7.

Advantages of the ViraPower™ Promoterless Lentiviral Gateway® Expression System

Use of the ViraPower™ Promoterless Lentiviral Gateway® Expression System to facilitate lentiviral-based expression of the gene of interest provides the following advantages:

- Allows production of a lentiviral construct that facilitates expression of a gene of interest under the control of a promoter of choice.
- Generates replication-incompetent lentivirus that effectively transduces both dividing and non-dividing mammalian cells, thus broadening the potential applications beyond those of traditional retroviral systems (Naldini, 1998).
- Efficiently delivers the gene of interest to mammalian cells in culture or *in vivo* (Dull *et al.*, 1998).
- Provides stable, long-term expression of a target gene beyond that offered by adenoviral-based systems (Dull *et al.*, 1998; Naldini *et al.*, 1996).
- Produces a pseudotyped virus with a broad host range (Yee *et al.*, 1994).
- The expression vector in the System is adapted with MultiSite Gateway® Technology for easy, simultaneous, recombination-based cloning of multiple DNA fragments in a defined order and orientation.
- Includes multiple features designed to enhance the biosafety of the system.

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Overview, continued

ViraPower™ Lentiviral Technology

The ViraPower™ Lentiviral Technology facilitates highly efficient, *in vitro* or *in vivo* delivery of a target gene or RNA to dividing and non-dividing mammalian cells using a replication-incompetent lentivirus. Based on the lentikat™ system developed by Cell Genesys (Dull *et al.*, 1998), the ViraPower™ Lentiviral Technology possesses features which enhance its biosafety while allowing high-level expression in a wider range of cell types than traditional retroviral systems. The main components of the ViraPower™ Lentiviral Expression System include:

- A pLenti-based expression vector into which the DNA sequence (or sequences) are cloned. This vector contains elements required to allow packaging of the expression construct into virions and an antibiotic resistance marker to allow selection of stably transduced cell lines. For more information, see page 5.
- The ViraPower™ Packaging Mix, an optimized mixture of the three packaging plasmids required for production of the lentivirus.
- A 293FT producer cell line to facilitate optimal production of virus.

For more information about the ViraPower™ lentiviral components in this kit, see page 4. For more information about the biosafety features of the System, see page 8.

Purpose of this Manual

This manual provides an overview of the ViraPower™ Promoterless Lentiviral Gateway® Expression System and provides instructions and guidelines to:

1. Generate entry clones containing the promoter and gene of interest, one in pENTR™5′-TOPO® and the second in any Gateway® entry vector (guidelines only provided).
2. Use the pLenti6/R4R2/V5-DEST vector and two entry clones containing the promoter and gene of interest in a MultiSite Gateway® LR recombination reaction to generate an expression clone.
3. Cotransfect the pLenti6/R4R2/V5-DEST expression construct and the ViraPower™ Packaging Mix into the 293FT Cell Line to produce a lentiviral stock.
4. Titer the lentiviral stock.
5. Transduce the mammalian cell line of choice with the Lenti6/R4R2/V5-DEST lentiviral construct.
6. Assay for "transient" expression of your recombinant protein or generate a stably transduced cell line, if desired.

For details and instructions to generate the entry clone containing the promoter of interest, refer to the pENTR™5′-TOPO® TA Cloning Kit manual. For instructions to generate the entry clone containing the gene of interest, refer to the manual for the entry vector you select. For instructions to culture and maintain the 293FT producer cell line, refer to the 293FT Cell Line manual. The pENTR™5′-TOPO® TA Cloning® Kit and 293FT Cell Line manuals are supplied with Catalog no. K5910-00. All manuals are available for downloading from www.invitrogen.com or by contacting Technical Support (see page 56).

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Overview, continued

Important

The ViraPower™ Promoterless Lentiviral Expression System is designed to help you create a lentivirus to deliver and express a gene of interest from a promoter of choice in mammalian cells. Although the system has been designed to help you express your recombinant protein of interest in the simplest, most direct fashion, use of the system is geared towards those users who are familiar with the principles of retrovirus biology and retroviral vectors. In addition, we highly recommend that users possess a working knowledge of:

- Viral and tissue culture techniques
- Gateway® Technology and site-specific recombination

For more information about these topics, refer to the following published reviews:

- Retrovirus biology and the retroviral replication cycle: see Buchschacher and Wong-Staal (2000) and Luciw (1996).
- Retroviral and lentiviral vectors: see Naldini (1999), Naldini (1998), and Yee (1999)
- Gateway® Technology and site-specific recombination: see Hartley *et al.* (2000) and Landy (1989)

Note

The One Shot® Stbl3™ Chemically Competent *E. coli*, LR Clonase™ II Plus Enzyme Mix, and Lipofectamine™ 2000 Reagent included in the ViraPower™ Promoterless Lentiviral Gateway® Expression System are available separately from Invitrogen and are each supplied with individual documentation detailing general use of the product. **For instructions to use these products specifically with the ViraPower™ Promoterless Lentiviral Gateway® Expression System, follow the recommended protocols in this manual.**

The ViraPower™ Promoterless Lentiviral Gateway® Expression System

Components of the ViraPower™ Promoterless Lentiviral Gateway® Expression System

The ViraPower™ Promoterless Lentiviral Gateway® Expression System facilitates highly efficient, lentiviral-based, *in vitro* or *in vivo* expression of a gene of interest under the control of a promoter of choice in dividing and non-dividing mammalian cells. The kit includes the following major components:

- The pENTR™5'-TOPO® TA Cloning Kit containing the pENTR™5'-TOPO® vector for production of an entry clone containing the promoter of interest. The vector is TOPO®-adapted and MultiSite Gateway®-adapted to allow TOPO® Cloning of a *Taq* polymerase-amplified PCR product encoding the promoter of interest and easy transfer of the promoter sequence into the pLenti6/R4R2/V5-DEST vector, respectively. For more information about the MultiSite Gateway® Technology, see page 6. For detailed information about the pENTR™5'-TOPO® vector and instructions to generate an entry clone, refer to the pENTR™5'-TOPO® TA Cloning® Kit manual.

  Important: To generate the pLenti6/R4R2/V5-DEST expression construct, you will also need to generate an entry clone containing your gene of interest. In this instance, you may use any standard Gateway® entry vector except pENTR™5'-TOPO®. For more information, see page 6.

- The pLenti6/R4R2/V5-DEST expression vector into which the promoter and gene of interest will be simultaneously cloned using MultiSite Gateway® Technology. The vector also contains the elements required for packaging of the expression construct into virions (*e.g.* 5' and 3' LTRs, ψ packaging signal) and the Blasticidin resistance marker to allow generation of stable cell lines. For more information about the pLenti6/R4R2/V5-DEST vector, see page 5.
- The ViraPower™ Packaging Mix that contains an optimized mix of the three packaging plasmids, pLP1, pLP2, and pLP/VSVG. These plasmids supply the helper functions as well as structural and replication proteins *in trans* required to produce the lentivirus. For more information about the packaging plasmids, see the **Appendix**, pages 50-55.
- An optimized 293FT producer cell line that stably expresses the SV40 large T antigen under the control of the human CMV promoter and facilitates optimal production of virus. For more information about the 293FT Cell Line, refer to the 293FT Cell Line manual.

After you have generated the pLenti6/R4R2/V5-DEST expression construct containing your promoter and gene of interest, you will cotransfect the plasmid and the ViraPower™ Packaging Mix into 293FT cells to produce a replication-incompetent lentiviral stock. This lentiviral stock may then be transduced into the mammalian cell line of interest to express your recombinant protein.

How Lentivirus Works

Once the lentivirus enters the target cell, the viral RNA is reverse-transcribed, actively imported into the nucleus (Lewis & Emerman, 1994; Naldini, 1999), and stably integrated into the host genome (Buchschacher & Wong-Staal, 2000; Luciw, 1996). After the lentiviral construct has integrated into the genome, you may assay for transient expression of your recombinant protein or use antibiotic selection to generate a stable cell line for long-term expression studies.

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The ViraPower™ Promoterless Lentiviral Gateway® Expression System, continued

VSV Envelope Glycoprotein

Most retroviral vectors are limited in their usefulness as gene delivery vehicles by their restricted tropism and generally low titers. In the ViraPower™ Promoterless Lentiviral Gateway® Expression System, this limitation has been overcome by use of the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) as a pseudotyping envelope, thus allowing production of a high titer lentivirus with a significantly broadened host cell range (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).

In vivo Gene Delivery

The ViraPower™ Promoterless Lentiviral Expression System is suitable for *in vivo* gene delivery applications. Many groups have successfully used lentiviral vectors to express a target gene in tissues including brain, retina, pancreas, muscle, liver, and skin (Gallichan *et al.*, 1998; Kafri *et al.*, 1997; Miyoshi *et al.*, 1997; Naldini, 1998; Pfeifer *et al.*, 2001; Pfeifer *et al.*, 2001; Takahashi *et al.*, 1999). For more information about target genes that have been successfully expressed *in vivo* using lentiviral-based vectors, refer to the references above as well as the following additional references (Baek *et al.*, 2001; Dull *et al.*, 1998; Lois *et al.*, 2002; Park & Kay, 2001; Peng *et al.*, 2001).

Features of the pLenti6/R4R2/V5-DEST Vector

The pLenti6/R4R2/V5-DEST vector contain the following elements:

- Rous Sarcoma Virus (RSV) enhancer/promoter for Tat-independent production of viral mRNA in the producer cell line (Dull *et al.*, 1998)
- Modified HIV-1 5′ and 3′ Long Terminal Repeats (LTR) for viral packaging and reverse transcription of the viral mRNA (Dull *et al.*, 1998; Luciw, 1996)

  Note: The U3 region of the 3′ LTR is deleted (ΔU3) and facilitates self-inactivation of the 5′ LTR after transduction to enhance the biosafety of the vector (Dull *et al.*, 1998)
- HIV-1 psi (Ψ) packaging sequence for viral packaging (Luciw, 1996)
- HIV Rev response element (RRE) for Rev-dependent nuclear export of unspliced viral mRNA (Kjems *et al.*, 1991; Malim *et al.*, 1989)
- Two recombination sites, *attR4* and *attR2* for recombinational cloning of the promoter and gene of interest from two separate entry clones
- The *ccdB* gene located between the *attR* sites for negative selection
- Chloramphenicol resistance gene (Cm^R) located between the two *attR* sites for counterselection
- C-terminal V5 epitope for detection of the recombinant protein of interest (Southern *et al.*, 1991)
- Blasticidin resistance gene for selection in *E. coli* and mammalian cells (Izumi *et al.*, 1991; Kimura *et al.*, 1994; Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965)
- Ampicillin resistance gene for selection in *E. coli*
- pUC origin for high-copy replication of the plasmid in *E. coli*

Biosafety Features of the System

Introduction

The lentiviral and packaging vectors supplied in the ViraPower™ Promoterless Lentiviral Gateway® Expression System are third-generation vectors based on lentiviral vectors developed by Dull *et al.*, 1998. This third-generation HIV-1-based lentiviral system includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. These safety features are described below.

Biosafety Features of the ViraPower™ Promoterless Lentiviral System

The ViraPower™ Promoterless Lentiviral Gateway® Expression System includes the following key safety features:

- The pLenti6/R4R2/V5-DEST vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee *et al.*, 1987; Yu *et al.*, 1986; Zufferey *et al.*, 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (*i.e. gag*, *pol*, and *rev*).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids (*i.e.* three packaging plasmids and pLenti6/R4R2/V5-DEST). All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull *et al.*, 1998).
- Although the three packaging plasmids allow expression *in trans* of proteins required to produce viral progeny (*e.g.* gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the *gag* and *pol* genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull *et al.*, 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti6/R4R2/V5-DEST vector to offset the requirement for Tat in the efficient production of viral RNA (Dull *et al.*, 1998).

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Biosafety Features of the System, continued

Biosafety Level 2

Despite the inclusion of the safety features discussed on the previous page, the lentivirus produced with this System can still pose some biohazardous risk since it can transduce primary human cells. For this reason, **we highly recommend that you treat lentiviral stocks generated using this System as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.** Furthermore, exercise extra caution when creating lentivirus carrying potential harmful or toxic genes (*e.g.* activated oncogenes).

For more information about the BL-2 guidelines and lentivirus handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories", 4th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded at the following address:

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

Important

Handle all lentiviruses in compliance with established institutional guidelines. Since safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or safety officer(s) at your institution prior to use of the ViraPower™ Promoterless Lentiviral Gateway® Expression System.