Modification Form for Permit BIO-LHRI-0083

Permit Holder: Ting Yim Lee

Approved Personnel
(Please stroke out any personnel to be removed)

Lisa Hoffman

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Lentivirus	
Approved Cells	[Primary] - (rodent): muscle satellite cells, primary myoblasts. [Established] - (human): LoVo, NCI-H1299, PC-3, HEK, 293T. (rodent): C2C12, C6.	
Approved Use of Human Source Material		
Approved GMO	[Vector] - ViraPower Promoterless Lentiviral Vector. [Plasmid] - Myogenin promoter, trifusion reporter, lipofectamine	
Approved use of Animals	Mouse (AUS 2008 - 067)	
Approved Toxin(s)	~~Cardiotoxin~~	Cardiotoxin - see attached MSDS

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2+

Date of last Biohazardous Agents Registry Form: Jul 23, 2009

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

→ Following the "Biosecurity Requirements for Facilities Using Biological Agents" is recommended (required on campus).

– 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 breathing apparatus, rubber boots and gloves. Sweep up. Do not raise dust. Wash and ventilate spill site 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 equipped 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. Avoid prolonged or frequent exposure. Wash thoroughly after handling.

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.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 2008
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: Dr. Ting-Yim Lee
SIGNATURE: Ting-Yim Lee
DEPARTMENT: Imaging
ADDRESS: The Lawson Health Research Institute
PHONE NUMBER: (x) 24131
EMAIL: tlee@lawsonimaging.ca

Location of experimental work to be carried out: Building(s) LHRI Room(s) F4-127a

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES:
GRANT TITLE(S):

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:
Lisa Hoffman

1.0 Microorganisms

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

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)? (see attached, ordered from InVitrogen)
Please describe the risk (if any) of escape and how this will be mitigated:
(see attached). Rooms in which lentivirus are Level 2+ (ie. Hepa-certified BSCs, portable autoclave, gloves, labcoats, 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)*	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	Health Canada or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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? ✓ 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	⊗ Yes O No		Not applicable
Rodent	✓ Yes O No	i) muscle satellite cells (SCs) harvested from transgenic mice (not a biohazard, Level 1, housed at Life;)	
Non-human primate	O Yes O No		
Other (specify)	O Yes O No	2) primary myoblasts (obtained from Dr. Michael Rudnicki, See attached reference)	

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4) HEK (also called 293) received from Dr. Greg Debelan, - human Kidney available from InVitrogen (see attached)

5) 293T (ATCC / human Kidney) (see attached notes)

] Level 2

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⊗ Yes O No	1 LoVo 2 NCI - H1299 3 PC-3	ATCC (colorectal adeno - carcinoma) ATCC / lung carcinoma ATCC / prostate adeno carcinoma
Rodent	⊗ Yes O No	1 C2C12 (mouse) 2 CC (rat)	ATCC / muscle carcinoma ATCC / glioma
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

Level 1
Level 1
Level 1
Level 1
Level 1

*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 HC or CFIA containment level required ⊗ 1 ⊗ 2 O 3

HEK/293T

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
Stbl3 competent E-coli	ViraPower Promoterless Lentiviral vector (InVitrogen)	InVitrogen	Myogenin Promoter - Trifusion Reporter - (Firefly Luciferase/ monomeric red fluorescent protein/ truncated sr39-thymidine Kinase)	Expression of the reporter genes for optical & PET imaging

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

→ Lipofectamine cationic lipid-based transfection reagent to be used (InVitrogen)

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
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 ⊘YES, please specify See attached sheet O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ⊘NO
- SV 40 Large T antigen ⊘YES O NO
- E1A oncogene O YES ⊘NO
- Known oncogenes O YES, please specify _____ ⊘NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ⊘NO

4.5 Will virus be replication defective? ⊘YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⊘NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus 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? ⊘YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used male mouse, nude/athymic mouse

6.3 AUS protocol # 2008-067

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES 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 O NO
- Others (wild or domestic) O YES, please specify _____ O NO

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)_____
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_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? 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 lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ✓NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the 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

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ✓YES, please give country of origin USA
If no, please proceed to Section 10.0 O NO

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

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

11.4 Has the import permit been sent to OHS? ✓YES, please provide permit # P-13043 O NO
A-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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus B10-LHR-0083
O NO
O NOT REQUIRED

14.0 Procedures to be Followed

14.1 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 Jun Lee Date: May 5 / 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kalra
Date: 23 July 2009

Safety Officer for Institution where experiments will take place: SIGNATURE:
Date: May 5 / 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: Jeff Tunley.
Date: July 13/09

Approval Number: B10 - LHR-0083 Expiry Date (3 years from Approval): July 22, 2012

Special Conditions of Approval:

- The Imaging component will need to follow an SOP similar to that for Robarts. More details will need to be provided to the committee before level 2+ (lentiviral vector) imaging work can be done - such as location(s) and SOP to be followed.
- Per permit P-13043, you may need to inform PHAC that you are using / transferring the agent imported ?

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

------- Original Message --------

Subject: Re: Dr. Lee Biohazardous Agents Registry Form
Date: Thu, 14 May 2009 13:39:39 -0400
From: Jeff Tucker <Jeff.Tucker@sjhc.london.on.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
References: <4A0B3427.3000900@uwo.ca>

Hi Jennifer:

He is an imaging scientist and I believe that the documentation submitted was for all of the agents his lab group would be using, partly as requested by yourself. All of the agents work is done by Savita's group since she has the culture room and expertise working with these agents.

I think part of the problem is that the form only has three choices 1,2 and 3. The level 2+ work would be related to the lentiviral kits and I believe this submission is just for culturing and not specifically for administration to animals. The administration part, as I understand from Jennifer Hadway is still being worked out in regard to the HEPA containment device.

Savita has a level 2+ approved lab with portable autoclave.

Jeff

------ Original Message --------

Subject: Re: Biohazardous Agents Registry Form: Dr. T. Lee
Date: Tue, 19 May 2009 13:31:33 -0400 (EDT)
From: Lisa Hoffman <lhoffman@lawsonimaging.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
References: <4A0DDC7A.8040009@uwo.ca>

Hi Jennifer,

As per your request, here is a brief description of the research to be conducted with the following cell lines:

1) C2C12 cells, muscle satellite cells (SCs) and primary myoblasts (all engineered to express our PET reporter gene): for transplantation into dystrophic mice; muscle repair/regeneration will be assessed post cell therapy.

2) HEK, 293T cells: our PET reporter construct will be introduced into these producer cell lines. The resulting supernatant will be used to infect cultured myoblasts.

3) C6 cells: used to create gliomas in nude rats

4) LoVo cells: used to create subcutaneous tumors in nude rate

5) NCI-H1299: used to create a lung carcinoma in mude mice

6) PC-3: used to creat prostate tumors in nude mice

I hope this will suffice. Please let me know if you require any additional
information.

Thanks,

Lisa

USE OF ROBARTS IMAGING SUITES: BIOSAFETY REQUIREMENTS FOR *IN VIVO* AND *IN VITRO* WORK

Approved: February, 2009
University of Western Ontario Biosafety Committee
(Original Approved February 13, 2008)

1.0 Introduction and Scope:

Imaging Facilities at Robarts are used for *in vitro* and *in vivo* work by researchers throughout London affiliated with the University of Western Ontario. The objective of this document is to ensure that this research meets the standards set by the latest versions of the Health Canada Laboratory Biosafety Guidelines, the Containment Standards for Veterinary Facilities by the Canadian Food Inspection Agency and, where animals are involved, the Canadian Council for Animal Care (CCAC). This work must also follow the Biosafety Guidelines and Procedures Manual found at: www.uwo.ca/humanresources/biosafety.

The goal of this document is to ensure that *in vitro* and *in vivo* experiments meet all applicable guidelines and regulations and are done within the proper containment to protect the work, the animals, the facilities, and the faculty, staff, and students who perform the work.

- This document applies to the 3T MRI, 9.4T Imaging Suite (MRI), Human High Field MRI Laboratory (3T & 7T), and Preclinical Imaging Suite (MicroCT, Ultrasound, SPECT CT) imaging facilities and includes procedures for transport to the facility. With respect to the primate facilities (including the 9.4T MRI suite), upon arrival at the facility the approved facility SOPs take effect.

- This document applies only to containment level 1 (CL1), level 2 (CL2) or level 2 with level 3 operations. Research requiring level 3 containment must contact the Biosafety Officer at biosafety@uwo.ca. Level 2 research involving live non-human primates must follow the Standard Operating Procedures (SOPs) for the Center for the Brain and Mind.

1.1 General Safety Precautions for *In vivo* and *In vitro* Imaging

- All personnel operating the imaging equipment (9.4T MRI, 3T MRI, 7T MRI, MicroCT, SPECT CT, and Ultrasound) must be trained by Facility Manager or designate.
- All personnel handling animals must have the required Animal Care and Veterinary Services training.

- All animal work must be outlined in an approved animal use protocol.

- All personnel using the Imaging facilities must be trained and follow the Standard Operating Procedures (SOPs) in place for each facility.

- Supervisors must ensure that people using the Imaging facilities have the appropriate health and safety training for the work being performed, per the Health and Safety Training found at: http://www.uwo.ca/humanresources/facultystaff/h_and_s/training/training_idx.htm

- Personnel using each Imaging facility must wear the appropriate personal protective equipment. For more information, see the Laboratory Safety Manual, www.uwo.ca/humanresources or contact the Lab Safety Coordinator.

- Disposal of waste, including hazardous chemical waste, biomedical waste, animal waste and carcasses, must follow the Hazardous Material Management Handbook: http://www.uwo.ca/humanresources/facultystaff/h_and_s/enviromental_prog/environmental_idx.htm

- Work carried out must meet the requirements of the Biosafety Guidelines and Procedures Manual found at: www.uwo.ca/humanresources/biosafety

- Personnel should complete their Hazard Communication Form and have the appropriate medical surveillance. For information, please see: http://www.wph.uwo.ca/newposition.htm.

- In case of an emergency, such as medical or fire, personnel follow the SOPs in place for the facility accessible on-line or in the Robarts Health and Safety Office.

  Preclinical Imaging Suite: SOP 900 -- Emergency Procedures
  9.4T MRI Facility: SOP 300 – Standard Operating Procedure: Emergency Fire Procedures
  3T MRI Facility: SOP 3T 215, 210, and 205 – Standard Operating Procedures for Emergency Quench, Fire Code Blue
  Human High Field MRI Lab: SOP 220, 230, and 210 – Emergency Fire, Emergency Quench, Emergency Code Blue

  Where there is an emergency involving human and animal wellbeing, human health and safety is the priority.

- The Principal Investigator must have an approved, current Biohazardous Agents Registry Form on file with the Biosafety Office which reflects the research being done. For more information, see: www.uwo.ca/humanresources/biosafety.

- The Biosafety Officer(s) in association with the Director, Animal Care and Veterinary Services and the Biohazard Subcommittee determine the containment level required for the work being performed.

1.2 Transportation of Animals

1.2.1 Transportation of Level 1 Rodents

Level 1 rodents are those not exposed to a CL2 (or higher CL) agent via ingestion, inhalation, injection, or absorption and are not known to carry a level 2 zoonotic agents. Level 1 rodents may be transported to the Robarts imaging facilities and within the Robarts building using standard cages. Level 1 rodents may be transported to the University or within the University buildings in standard cages.

1.2.2 Transportation of Level 2 Rodents

Level 2 rodents are those which have been exposed to a CL2 agent. Level 2 rodents must be transported in a HEPA-filtered cages or an apparatus. The cages or apparatus must be approved by the Director, ACVS and the Biosafety Officer(s) for Robarts. The transportation of level 2 animals by road, rail, water or air must also follow the appropriate transportation of dangerous goods regulations.

1.2.3 Transportation of Non Human Primates (NHP)

Transportation of non human primates is governed by a separate set of SOPs that have been approved by ACVS, members of the Centre for Brian and Mind, and the Biosafety Officers for Robarts. These SOPs are available in the Brain and Mind Facility or the Robarts Health and Safety office and are to be followed for the transportation of primates (NHP) to and from the primate (NHP) quarters and the MRI suites.

2.0 Introduction to Rodent and Non Human Primate (NHP) Imaging Research

Animal projects must be approved by the Animal Use Subcommittee of the University Council on Animal Care. Animals are housed in areas approved by Animal Care and Veterinary Services (ACVS) and the Canadian Council on Animal Care (CCAC). Animals are transported to the facility in cages on carts.

2.1 Imaging Involving Level 1 Rodents

- Level 1 rodent work involves rodents that have not been exposed to a level 2 (or higher CL) agent via ingestion, inhalation, injection or absorption and that are not known to carry a level 2 (or higher CL) zoonotic agent. An example of a level 1 rodent is an animal procured from a commercial supplier or one injected with a murine pathogen free cell line approved by Biosafety at level 1.

2.1.1 Safety Precautions

- Follow the Standard Operating Procedure (SOPs) for the decontamination of samples entering the facility and the clean-up of animal excrement, including surface disinfection. Disinfectants

must be approved by the Biosafety Officer or in the SOP and must be effective and safe to use on the equipment. The SOPs are available on-line or in the Robarts Health and Safety Office.

Third Floor Preclinical Imaging Suite: SOP 500 – Cleaning and Decontamination
First Floor 9.4T MRI Facility: SOP 415 – Cleaning and Disinfection – Level 1 & 2 Experiments
Second Floor 3T MRI Facility: SOP 400 – Standard Operating Procedure for MRI Decontamination
First Floor Human High Field MRI Lab: SOP 415 – Cleaning and Decontamination – Level 1 & 2 Experiments

- Gloves and other personal protective equipment must be changed if they have been in contact with animal wastes.

- Procedures such as injections, surgery, anesthesia, and euthanasia can be done on the open bench. Scavenging devices must be used in association with anesthesia or euthanasia with a gaseous agent. If a hazardous chemical or radioactive material is involved, this may require the use of a fume hood elsewhere and additional precautions/approvals.

- The animal may be placed in the coil or bed on the open bench.

- In case of a veterinary emergency, life-saving procedures can be done on the open bench.

2.2 Imaging Involving Level 2 Rodents

- Level 2 Rodent work involves animals that have been exposed to a level 2 agent via ingestion, inhalation, injection or absorption or carry a level 2 zoonotic agent. Examples of level 2 pathogens include:

    - Viral vectors such as adenovirus and retroviruses
    - Human cell lines such as HEK293, which carries an activated human oncogene, or non-human primate cell lines such as cos-7, because they may carry viruses capable of infecting humans
    - Microorganisms such as Salmonella sp. or Pseudomonas sp.
    - Biological toxins such as pertussis and cholera toxin.

Contact the Biosafety Officer at biosafety@uwo.ca for the containment level of the project. For more information, please see www.uwo.ca/humanresources/biosafety

2.2.1 Safety Precautions

For level 2 projects, there are additional Safety Precautions to those in Section 2.1.1.

- Level 2 agents must be handled in a Class 2 biological safety cabinet. Animals that have been exposed to a level 2 agent must be kept in an approved HEPA-filtered cage or apparatus during the duration of the experiment, including housing, transportation, imaging and during veterinary life saving measures.
- Personnel using an approved HEPA-filtered cage or apparatus must have a plastic container with them. In case of failure or leakage of the cage or apparatus, the cage or apparatus (with the animal inside) is put in the plastic container. The container can only be opened in a biological safety cabinet.
- Animals exposed to a level 2 agent must be housed in a certified ACVS approved level 2 housing facility.

2.2.1.1 Preclinical Imaging Suite and Second Floor 3T MRI Facilities

Personnel can transport the animals in a HEPA-filtered cage to the imaging facility. The cage must be opened in the biological safety cabinet to perform procedures such as injections, anesthesia and veterinary life saving measures. The animal is placed in a HEPA-filtered apparatus for imaging in the biological safety cabinet. After imaging, the rodent is transported to a biological safety cabinet in an approved level 2 housing facility. The apparatus is never opened except in a biological safety cabinet.

The apparatus must be certified by a certified contractor such as HEPA Filters Inc. The apparatus must be approved by the Biosafety Officers for Robarts and Animal Care and Veterinary Services. The apparatus must maintain level 2 containment, and requires safety features such as HEPA filtration, O-rings, threaded ends.

HEPA-filtered cages must be approved by the Biosafety Officers for Robarts and by Animal Care and Veterinary Services.

Waste is collected from the biological safety cabinet in bags. The bag is closed in the biological safety cabinet and disposed of by the research personnel. Carcasses are disposed of by research personnel. Waste is disposed of per the Hazardous Materials Management Handbook.

2.2.1.2 9.4T MRI Facility and Human High Field MRI Laboratory (3T & 7T)

2.2.1.2.1 Approach #1

This facility does not contain a biological safety cabinet. Procedures must be done in a biological safety cabinet in an approved level 2 facility elsewhere.

Animals must be placed in an approved HEPA-filtered imaging apparatus in a biological safety cabinet in an approved level 2 laboratory. Animals are transported to the facility and imaged in this apparatus. The apparatus is never opened except in a biological safety cabinet.

Waste is collected in autoclaveable bags and disposed of by the research personnel. Carcasses are also disposed of by research personnel. Waste is disposed of per the Hazardous Materials Management Handbook.

The apparatus must be certified by a certified contractor such as HEPA Filters Inc. The apparatus must be approved by the Biosafety Officers for Robarts and Animal Care and Veterinary Services. The apparatus must maintain level 2 containment, and requires safety features such as HEPA filtration, O-rings, threaded ends.

2.2.1.2.2 Approach #2

In some cases, approach #1 is impractical; approach #2 can then be used for level 2 rodents. This is based on a case-by-case risk assessment and is approved by the Biosafety Officers for Robarts and Animal Care and Veterinary Services.

When the rodents have been previously exposed to a level 2 agent, they are brought to the MRI facilities for imaging using an approved HEPA-filtered transport cage on a cart and placed in the appropriate imaging insert coils.

Approach #2 for MRI and fiber optic imaging of level 2 animals in the MRI suites is based on designing and constructing the whole lab to be under level 2 containment. This means that the air entering and leaving the MRI suites is HEPA-filtered. Entrance is through a controlled air lock and the room is under negative air pressure to the adjacent corridor. Personnel must wear the appropriate personal protective equipment as mandated by the MRI Facility's SOP 210-01. This includes the wearing of a fit-tested N95 respirator when working with level 2 animals as a biological safety cabinet is not available. Protective clothing must be removed before leaving the MRI facilities

as stated in SOP 210. Decontamination procedures for the suites are outlined in the Facility's SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 for the Center for Brain and Mind. Personnel must be specially trained to work in the MRI level 2 containment suites.

Waste is collected in autoclaveable bags and disposed of by the research personnel. Carcasses are also disposed of by research personnel. Waste is disposed of per the Hazardous Materials Management Handbook.

2.2 Imaging Involving Non-Human Primates

Approach #2 for MRI and fiber optic imaging of level 2 animals in the MRI suites is based on designing and constructing the whole lab to be under level 2 containment. This means that the air coming in and leaving the MRI suites is HEPA-filtered. Entrance is through a controlled air lock and the room is under negative air pressure to the adjacent corridor. Personnel must wear the appropriate personnel protective equipment as mandated by the MRI Facility's SOP 210-01. This includes the wearing of a fit-tested N95 respirator when working with level 2 animals as a biological safety cabinet is not available. Protective clothing must be removed before leaving the MRI facilities as stated in SOP 210. Decontamination procedures for the MRI suites are outlined in the Facility SOP 415 and the MRI Suite Decontamination procedures for the suites are outlined in the Facility's SOP 415 and the MRI Suite Decontamination Procedures: SOP 3900 for the Center for Brain and Mind. Researchers must follow the Use of MRI Suite for NHP Imaging: SOP 4600 and other Center for Brain and Mind Rhesus Facility Standard Operating Procedures. Personnel must be specially trained to work in the MRI level 2 containment suites.

3.0 Introduction to *In vitro* Research Involving Imaging

Samples are prepared for imaging in an approved biosafety laboratory. Samples are brought to the imaging facility in sealed leak- and shatter-proof containers. Samples are put in a coil or a bed and/or HEPA-filtered apparatus for imaging purposes.

3.1 Imaging Involving Fixed Samples

Level 2 or level 2+3 samples fixed with chemicals such as formalin or comparable agent are no longer considered biohazardous. These samples can be imaged as level 1 samples. If samples need to be opened, they should be opened in a chemical fume hood.

3.2 Imaging Involving Level 1 *In Vitro* Work

Samples must be transported to the facility in sealed leak- and shatter-proof containers. Containers must be wiped off with a disinfectant before they leave the laboratory and per the SOPs for the facility. Work with these samples can be done on the open bench, providing that no hazardous chemicals are involved. If hazardous chemicals or radioactive materials are involved, work must be done in a fume hood elsewhere and additional precautions/approvals are required.

3.3 Imaging Involving Level 2 *In vitro* Work

For level 2 projects, there are additional safety precautions to those in 3.1. Samples must be worked with using a biological safety cabinet.

3.3.1 Preclinical Imaging Suite and 3T MRI Facilities

If required, samples can be opened under the biological safety cabinet provided.

3.3.2 9.4T MRI Facility and Human High Field MRI Laboratory (3T & 7T)

There are no biological safety cabinets in these facilities. Samples must be prepared in a biological safety cabinet in an approved level 2 laboratory elsewhere. Sealed leak- and shatter-proof containers are not to be opened in the facilities. The sample is kept closed during transportation and imaging of the samples.

4.0 Imaging Involving Work at Level 2 plus Level 3 Operations

The researcher must have an approved, current Biohazardous Agents Registry Form on file with the Biosafety Office which reflects the research being done. For more information, see: www.uwo.ca/humanresources/biosafety

Certain projects, such as some research involving lentiviral-based vectors, require level 2 plus level 3 operations. For level 2 plus level 3 projects there are additional safety precautions. All work must be carried out in a biological safety cabinet.

4.1 Imaging

- Use portable autoclave to decontaminate waste prior to leaving the imaging facility. Follow the "SOP for the Sanyo Portable Autoclave".
- Injections must be done in the approved level 2 plus 3 laboratory or the level 3 facility on DSB, 6th floor.
- Animals transported on a cart to or within Robarts for imaging must be in a HEPA-filtered cage unit approved by Biosafety and ACVS.
- The cages can be removed from the transport cart and placed in a biological safety cabinet. Animals must be placed in an approved HEPA-filtered imaging apparatus (see section 2.2.1.1) in a biological safety cabinet in an approved level 2 plus 3 laboratory. Animals are transported to the facility and imaged in this apparatus. The apparatus is never opened except in a biological safety cabinet.
- After scanning, all reusable material (i.e. forceps) must be decontaminated in a Wescodyne solution in a biological safety cabinet. The Wescodyne working solution has: 40% H_2O, 40% ethanol and 20% Wescodyne. It can be prepared in advance.
- Submerse all the reusable instruments (surgical) in the labelled Wescodyne solution for 2 hours.
- Rinse the instruments after 2 hours with H_20 and let dry.

- After drying, pack in autoclave bags and sterilize in the portable autoclave (this is done to ensure successful sterilization).
- The procedures for disinfection of contaminated animal cages and bedding must be completed. Bedding must be emptied into a biohazard bag inside of the biosafety cabinet. The bedding must be then double bagged and sealed inside a biological safety cabinet. The bag must be wiped with a disinfectant before it is removed from the biological safety cabinet for disposal per the Hazardous Materials Management Handbook.
- Inside the hood, to the empty cage add Wescodyne solution and swirl to ensure contact of all surfaces. Wipe the cage lid with Wescodyne as well and ensure contact for 2 hours (either leave the cage in a dunk tank for 2 hours or put the wet cage into an autoclave bag and leave in the hood for 2 hours). Drain the Wescodyne and return the cages and lids for washing and packing to be autoclaved. Follow the procedures for the facility where the cages came from (ACVS or Robarts barrier facility).
- All sharps must be disposed of in a sharps container within the biosafety cabinet. The container must be wiped on the outside with the Wescodyne solution. The containers are then sent to the incinerator.
- All waste must be labelled appropriately before it is taken for disposal.
- After the scan the rodent/animal must be returned to the biological safety cabinet before it is removed from the HEPA-filtered apparatus and then it can be returned to its cage.
- Disposable personal protective equipment, such as gloves, must be put in an autoclaveable biohazard bag leaving the room.
- Wescodyne solution can be treated as hazardous waste after use per the Hazardous Waste Management Handbook: http://www.uwo.ca/humanresources/docandform/docs/ohs1/manuals/hazardous_handbook.pdf.

Please Remit To:

University of Western Ontario
Financial Services
Accounts Receivable Office
Support Services Building, Suite 6100
London, ON N6A 3K7

Page:	1
Invoice No:	T342356
Invoice Date:	12/19/2008
Customer Number:	WES001379
Payment Terms:	On Receipt
Due Date:	12/19/2008

Bill To:

Healthcare Materials Mgmt Serv-SJHC
Anna Ivanisevic
Accounts Payable
LHSC-UC
London ON N6A 4V2
Canada

Ship To:

AMOUNT DUE: $ 120.00 CAD

REMITTANCE ADVICE **PLEASE RETURN WITH PAYMENT** $ _____ Amount Remitted

INVOICE

Line	Description	Quantity	Unit of Measure	Unit Price	Amount	GST/ HST	PST
	Dhanvantari						
1	Inv 12855	1.00		120.00	120.00		

TOTAL AMOUNT DUE : 120.00 CAD

Billing Inquiries - 519-661-2111 EXT82656

Payment Inquiries - contact Accounts Receivable - Phone: 519/661-3870 Fax: 519/661-3829 Email: fin-aroffice@uwo.ca

Invoice Number:	T342356	Issued By:	Kathleen Perry	Page:	1
Invoice Date	12/19/2008	Issuing Unit:	Animal Care & Vet Services	Payment Terms:	On Receipt
Customer Number:	WES001379	Phone Number	519-661-2111 EXT82656	1.50% per month on overdue accounts	
Purchase Order Number:		Fax Number:	519/661-2028		
Contract Number:		Bill Type:	003		
GST/Business Number:	10816 2587 RT 0001			Amount Remitted: $_____	

Western

University of Western Ontario
Financial Services
Accounts Receivable Office
Support Services Building, Suite 6100
London ON N6A 3K7

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 no.-Permis no.

P-[illegible]

Permit to import human pathogen(s)

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

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 DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
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

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

P-13043

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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
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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 DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR
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 requirement(s) 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.

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	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 1 fees.

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

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

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

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

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

nporter/Importateur	Exporter/Exportateur
AWSON RESEARCH INSTITUTE 68 GROSVENOR STREET, ROOM H417 ONDON, ONTARIO 6A4V2 ontact: Dr. Savita Dhanvantari Applicant Name. DR. SAVITA HANVANTARI 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)

ıd 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 ıat 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.

Cinthia Labrie

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) 263-5236
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 :

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

(Continue)

Telephone Number

THESE GOODS ARE FOR RESEARCH ONLY,UNLESS OTHERWISE SPECIFIED SEE "AUTHORISED USERS" IN GENERAL TERMS AND CONDITIONS 1
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

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

TO

LAWSON RESEARCH INSTITUTE
FOR Dr. Dhanvantari/Lisa Hoffman

LONDON
ON N6A 4V2 Canada
ATTN Lisa Hoffman

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 within 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.

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

Continued on next page

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.

Continued on next page

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.

continued on next page

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).

continued on next page

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.

continued on next page

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, *att*R4 and *att*R2 for recombinational cloning of the promoter and gene of interest from two separate entry clones
- The *ccd*B gene located between the *att*R sites for negative selection
- Chloramphenicol resistance gene (Cm^R) located between the two *att*R 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).

continued on next page

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.

Cell Biology

ATCC® Number:	**CRL-11268™** Order this Item	Price:	**$264.00**
Designations:	293T/17 [HEK 293T/17]	Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	*Homo sapiens* (human)	Morphology:	epithelial
Source:	**Organ:** kidney		
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.		

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Related Cell Culture Products

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]
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
Protocol:

Subculturing:

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

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: Every 2 to 3 days
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

derivative:ATCC CRL-11269
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361
57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999
57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001
57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

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Cell Lines

ATCC® Number: CCL-107 ™ Order this item **Price: $185.00**

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
Cell type: glial cell
Disease: glioma

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.

Related Cell Culture Products

Receptors:	glucocorticoid
Virus Susceptibility:	vesicular stomatitis (Indiana); vaccinia; herpes simplex
Virus Resistance:	poliovirus 3
Reverse Transcript:	negative
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:** Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 82.5%; horse serum, 15%; fetal

	bovine serum, 2.5% **Temperature:** 37.0C **Atmosphere:** air, 95%; carbon dioxide (CO2), 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 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' BSS (w/o Ca++, Mg++): ATCC 30-2101 Cell culture tested DMSO: ATCC 4-X
References:	1022: Benda P , et al. Differentiated rat glial cell strain in tissue culture. Science 161: 370-371, 1968. PubMed: 4873531 25965: Lightbody JJ , et al. Establishment of differentiated clonal strains of glial brain cells in culture. Fed. Proc. 27: 720, 1968. 32720: Chen Y , et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005

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Cell Lines

ATCC® Number: CCL-229 ™ Order this item **Price: $185.00**

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 **Disease:** colorectal adenocarcinoma **Tumor stage:** Dukes' type C, grade IV **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.		

Related Cell Culture Products

Tumorigenic:	Yes, in nude mice (Tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells)
Reverse Transcript:	negative
Oncogene:	myc +; myb + ; ras +; fos +; p53 +; sis -; abl -; ros -; src -
Antigen Expression:	HLA A11, B15, B17, Cw1, Cw3; blood type B
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:** Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 90%; fetal bovine serum, 10% **Temperature:** 37.0C
Subculturing:	Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation ratio:** A subcultivation ratio of 1:3 to 1:10 is recommended **Medium renewal:** 2 to 3 times per week
Preservation:	culture medium 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2004 recommended serum: ATCC 30-2020
References:	1047: Drewinko B , et al. Further biologic characteristics of a human carcinoembryonic antigen-producing colon carcinoma cell line. J. Natl. Cancer Inst. 61: 75-83, 1978. PubMed: 276641 1048: Drewinko B , Yand LY . Restriction of CEA synthesis to the stationary phase of growth of cultured human colon carcinoma cells. Exp. Cell Res. 101: 414-416, 1976. PubMed: 964319 1049: Drewinko B , et al. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res. 36: 467-475, 1976. PubMed: 1260746 22861: Trainer DL , et al. Biological characterization and oncogene expression in human colorectal carcinoma cell lines. Int. J. Cancer 41: 287-296, 1988. PubMed: 3338874 23341: Keesee SK , et al. Nuclear matrix proteins in human colon cancer. Proc. Natl. Acad. Sci. USA 91: 1913-1916, 1994. PubMed: 8127905 26057: Drewinko B , et al. Response of exponentially growing, stationary-phase, and synchronized cultured human colon carcinoma cells to treatment with nitrosourea derivatives. Cancer Res. 39: 2630-2636, 1979. PubMed: 445465 32913: Miranda L , et al. Isolation of the human PC6 gene encoding the putative host protease for HIV-1 gp160 processing in CD4+ T lymphocytes. Proc. Natl. Acad. Sci. USA 93: 7695-7700, 1996. PubMed: 8755538

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Cell Lines

ATCC® Number: CRL-1772 ™ Order this item

Price: $185.00

Designations: C2C12

Depositors: B Paterson

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: fibroblast PHOTO

Source: **Tissue:** muscle
Cell type: myoblast; 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.

Related Cell Culture Products

Strain:	C3H
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:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 90%; fetal bovine serum, 10% **Temperature:** 37.0C
Subculturing:	**Protocol:** IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT. Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture. Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

	1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2. 6. Incubate cultures at 37°C. **Medium renewal:** Every two to three days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2002 recommended serum: ATCC 30-2020
References:	22903: Yaffe D , Saxel O . Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524 22953: Blau HM , et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846 23427: Katagiri T , et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [published erratum appears in J Cell Biol 1995 Feb;128(4):following 713]. J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324 28236: Chow YH , et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336 32828: Kessler PD , et al. Gene delivery to skeletal muscle results in sustanined expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064 33069: Hsu DK , et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

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Cell Biology

ATCC® Number: CRL-5803™ Order this Item

Designations: NCI-H1299

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

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 tra of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the per Please click here for information regarding the specific requirements for shipment to your location.

Price: **$264.00**

Depositors: AF Gazdar, JD Minna

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

Related Cell Culture Prod

Restrictions: The line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Ga and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor products derived fr may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purpos sale, or producing for sale, cells or their products. The cells are provided as service to the research commu They are provided without warranty of merchantability or fitness for a particular purpose or any other warr expressed or implied. 2. Any proposed commercial use of the these cells, or their products must firs negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., D. Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.

Applications: transfection host (technology from amaxa
Roche FuGENE® Transfection Reagents)

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. reported to be able to synthesize the peptide neuromedin B (NMB) at 0.1 pmol/mg protein, but not the ga releasing peptide (GRP).

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Mec Catalog No. 30-2001. To make the complete growth medium, add the following components to the medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all trac 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 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 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 1:3 to 1:6 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 serum:ATCC 30-2020
purified DNA:ATCC CRL-5803D
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATC 2001

References:
23517: Giaccone G, et al. Neuromedin B is present in lung cancer cell lines. Cancer Res. 52: 2732s-2 1992. PubMed: 1563005
23570: . NCI-Navy Medical Oncology Branch Cell Line Supplement. J. Cell. Biochem. suppl. 24: 1996..
33177: Lin DL, Chang C. p53 is a mediator for radiation-repressed human TR2 orphan receptor express MCF-7 cells, a new pathway from tumor suppressor to member of the steroid receptor superfamily. J. Chem. 271: 14649-14652, 1996. PubMed: 8663350

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Cell Biology

ATCC® Number: CRL-1435™ Order this Item

Designations: PC-3

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

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 tra of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the per Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Prod

Applications: transfection host (technology from amaxa 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 chromos commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No norn chromosomes could be detected by Q-band analysis.

Age: 62 years adult

Gender: male

Price: **$256.00**

Depositors: ME Kaighn

Shipped: frozen

Growth Properties: adherent (The cells form clusters in soft agar and be adapted to suspensior growth)

Morphology: epithelial

Ethnicity: Caucasian

Comments: The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old 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 Me Catalog No. 30-2004. To make the complete growth medium, add the following components to the 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 trac 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 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 to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATC 2004
recommended serum:ATCC 30-2020

References:
22363: Kaighn ME, et al. Establishment and characterization of a human prostatic carcinoma cell line (I Invest. Urol. 17: 16-23, 1979. PubMed: 447482
22470: Chen TR. Chromosome identity of human prostate cancer cell lines, PC-3 and PPC-1. Cytogenet Genet. 62: 183-184, 1993. PubMed: 8428522
26302: Ohnuki Y, et al. Chromosomal analysis of human prostatic adenocarcinoma cell lines. Cancer Re 524-534, 1980. PubMed: 7471073
32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammar prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194
32344: Umekita Y, et al. Human prostate tumor growth in athymic mice: inhibition by androgen stimulation by finasteride. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996. PubMed: 8876218
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32486: Nupponen NN, et al. Genetic alterations in prostate cancer cell lines detected by comparative ge hybridization. Cancer Genet. Cytogenet. 101: 53-57, 1998. PubMed: 9460501
32488: Geiger T, et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with sta chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug De 35-45, 1998. PubMed: 9474241
32916: Su ZZ, et al. Surface-epitope masking and expression cloning identifies the human prostate carci tumor antigen gene PCTA-1 a member of the galectin gene family. Proc. Natl. Acad. Sci. USA 93: 7252- 1996. PubMed: 8692978

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