The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

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

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

PRINCIPAL INVESTIGATOR:	**Michael Jackson**
DEPARTMENT:	**Robarts Research Institute/ Physiology and Pharm**
ADDRESS:	**100 Perth Drive**
PHONE NUMBER:	**24230**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**mjacks32@uwo.ca**

Location of experimental work to be carried out :

Building : **RRI** Room(s): **7260C-1**
Building : Room(s):
Building : Room(s):

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

FUNDING AGENCY/AGENCIES: **Heart and Stroke Foundation of Ontario / CIHR**

GRANT TITLE(S): **Endoplasmic reticulum stress, Ca2+ permeation pathways and neurotoxicity / Cascades of Non-selective cation channels that mediate cell signaling or cell death in the Hippocampus**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

Name	UWO E-mail Address	Date of Biosafety Training
Natalie Lavine	**nlavine@uwo.ca**	**15/05/2009**
Ankur Bodalia	**abodalia@uwo.ca**	**21/09/2010**
Brian Lockhart	**blockha@uwo.ca**	**15/10/2010**
Gang Lei	**glei@uwo.ca**	**16/09/2009**

Jillian Belrose	jrobe55@uwo.ca	12/03/2010
Kai Yang	kyang43@uwo.ca	21/05/2009
Kristen Condie	kcondie@uwo.ca	23/06/2011
Lidia Brandes	lbrandes@uwo.ca	13/03/2009
Matt Johnston	mjohn45@uwo.ca	10/05/2010
Meng Tian	mtian9@uwo.ca	registered 18/07/12
Yu-Feng Xie	yxie47@uwo.ca	19/01/2009

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

Our major interest is in studying ion channel function and regulation, mainly TRP channels and Pannexin channels, in the context of understanding human physiology and disease. As part of our studies, we utilized 3 main experimental preparations; A) brain tissue acutely prepared from rodent, B) primary neuronal culture, prepared from rodent brain tissue and C) established cell lines (e.g. HEK 293). The biohazards listed are used to accomplish three broad experimental goals; 1) overexpression, 2) knockdown or 3) mutagenesis of a protein(s) of interest in a cell line or in our primary cultured neurons. Some specific examples of how the listed biohazards are utilized include: 1) Transfection of HEK 293 cells (listed in section 2.3) with plasmids (listed in section 4.2) allowing the overexpression of wild type (and mutant) pannexin channels for study in a simplified system. 2) Knockdown of pannexin channels by infection of primary cultured neurons with VSVG pseudotyped lentivirus (listed in section 1.2) packaged with a plasmid (listed in section 4.3) allowing for the expression of specific shRNA sequences targeting Panx1 subunit transcripts. 3) Transient transfection (or lentiviral-mediated infection) of primary cultured neurons with plasmids expressing dominant negative mutant forms of specific genes of interest (typically elements of intracellular signaling pathways that regulate surface expressed Ca2+ permeable channels).

Storage of Lentivirus:

All Lentivirus preparations are aliquoted in 25ul (or less) in leakproof cryovials, placed in freezer boxes, and stored in a locked freezer at -80C in Room 7250A. Detailed inventories of Lentivirus aliquots are available in a binder in Room 7260C-1.

Decontamination and disposal procedures:

All materials that come in contact with viral particles must be properly decontaminated prior to disposal.

1. Disposal/decontamination of solid waste such as, paper tissues, pipette tips, etc.: All solid waste (including disposable plastic wares) should be discarded in biohazard bags for the appropriate treatment (autoclaving) according to institutional practices and guidelines prior to disposal.
2. Disposal/decontamination of liquid waste: All liquid materials (Lentivirus-containing media, buffers, washes) are decontaminated inside safety cabinet by addition of Wescodyne Solution (20% Wescodyne/40% ethanol/40% water) prior to autoclaving.
3. Work surfaces inside cabinets should be decontaminated with Wescodyne Solution, followed by 70% ethanol and UV irradiation.
4. Instruments, equipment and any other items that are not disposable and come into contact with Lentivirus are bleached and/or autoclaved.

5. Routine laboratory cleaning will be done by lab personnel within the containment room.

Please see attched appendix for a more detailed description of our use and hanlding of lentivirus.

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

Stroke is a leading cause of death and disability. Over 50,000 Canadians suffer a stroke each year. In most patients, the stroke is caused by a blood clot interrupting the flow of blood to the brain. The only existing therapeutic option for stroke is the clot busting drug tPA (tissue plasminogen activator). However, tPA is only effective if given within 3-4.5 hours after stroke onset and most patients are admitted well beyond this time frame. As a result, less than 6% of stroke patients receive tPA treatment. This leaves the remaining population without recourse and the prognosis for these patients is bleak. Each year 14,000 Canadians die from stroke and many more (up to 75% of stroke sufferers) are left permanently disabled (disability ranging from minor to severe). If left untreated, the average stroke patient will lose 1.9 million neurons every minute. These facts underscore the urgent need for research seeking to identify novel therapies capable of protecting brain cells during a stroke (neuroprotection). One of the key indicators a neuron is about to die is when it begins to accumulate calcium. Indeed, while the concentration of calcium outside of brain cells is high, that within the intracellular environment is very low. During a stroke excessive quantities of calcium enter the cell through specialized proteins called channels. One type of channel, called the pannexin channel, has been shown to cause calcium entry during stroke. The objective of my project is to understand why pannexin channels open during stroke and how we can prevent this from happening. Ultimately, we wish to develop a novel neuroprotective agent for the treatment of stroke.

We will explore how pannexin channels are regulated using a well characterized cell model. Similar to a stroke, the model involves depriving cultured neurons of oxygen and nourishment. We will use genetic tools to disrupt the expression of candidate proteins responsible for provoking pannexin channel opening during a stroke. We will then determine whether this provides neuroprotection.

Although implicated in neuronal injury during stroke, the mechanisms responsible for promoting pannexin opening have yet to be identified. Our preliminary findings have identified a candidate protein capable of causing pannexin channel opening in neurons under conditions relevant to those that occur during a stroke. By understanding how exactly this occurs, we will be able to interrupt this process, prevent pannexin opening and thereby prevent the death of neurons during a stroke.

A single therapeutic option (tPA) is currently available for the treatment of stroke patients. Due to its limited therapeutic window, few patients currently receive tPA. The identification of previously unrecognized contributors to cell death during stroke, uncovered through our research, will allow for the rationale design of novel neuroprotective drugs.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species __________
What is the origin of the microorganism(s)? __________

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
VSVG pseudotyped lentivirus	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**0.04L**	**academic**	☐ 1 ☐ 2 ☒ 2+ ☐ 3
E.coli bacteria (DH5alpha, Rosetta-blue)	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.1ml**	**academic**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

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

Additional Comments: __________

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☒ No		Not applicable
Rodent	☒ Yes ☐ No	**mouse brain**	**2009-002**
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**Hek293(T)**	**2**	**ATCC**
Rodent	☐ Yes ☒ No			
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
DH5alpha Rosetta-blue	**pCDNA3.1, pGEX, pTNT**	**Invitrogen GE Healthcare Promega**	**TRP channels Pannexin channnels STIM proteins**	**no**	**no**	**amplification of the plasmid and protein expression**

E.coli

Sheet or equivalent if available.

uired for the following strains of E. coli:
andform/docs/ohs/CFIA_Ecoli_list.pdf

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Lentivirus	**pLB, FUGW, pCgpV, pCMV-Eco, pRSV-Rev**	**Addgene, Cell Biolabs Inc.**	**shRNA (for knockdown of TRPM2, Panx1, Panx2, STIM1, STIM2 and Scrambled sequence), GFP, mCherry**	**protein knockdown, protein expression for infection marker**

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

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

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

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

5.0 Will genetic sequences from the following be involved?

- HIV ☐ NO ☒ YES, specify **GAG-POL, RRE, ENV, REV**
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☐ NO ☒ YES
- E1A oncogene ☐ NO ☒ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

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

Additional Comments: _____

6.0 Human Gene Therapy Trials

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

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

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

7.2 Name of animal species to be used **Mouse**

7.3 AUS protocol # **2009-002**

7.4 List the location(s) for the animal experimentation and housing. **Medical Science Building**

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

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

8.0 Use of Animal species with Zoonotic Hazards

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

8.2 Will live animals be used? ☐ YES ☐ NO

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

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

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

9.0 Biological Toxins and Hormones

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

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

9.3 What is the LD_{50} (specify species) of the toxin or hormone **8-10ug/kg**

9.4 How much of the toxin or hormone is handled at one time*? **1 mg**

9.5 How much of the toxin or hormone is stored*? **5 mg**

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☒ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

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

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree…)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

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

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☒ YES, country of origin **tetrodotoxin imported from Israel** ☐ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☒ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☒ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☒ NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Michael Jacobs ***Date:*** 06/06/2012

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
- ☒ YES, location and date of most recent biosafety inspection: **August 31st, 2011**
- ☐ NO, please certify
- ☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

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

Rinse a minimum of 10 minutes in eye wash or flush with water and soap. For needlestick, cut, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name: Michael Madison ***Date:*** 06/06/2012

15.4 Additional Comments: _____

16.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Ronald Nasserden
Date: June 08, 2012

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

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

E. coli

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

Cinthia Labrie

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

Canada

Novagen®

Material Safety Data Sheet

Order Number **Customer Number**

1. Product and Company Identification

Supplier

Manufactured by EMD Biosciences, Inc.
441 Charmany Drive
Madison, WI 53719
(608)238-6110
(800)207-0144
FAX: (608)238-1388

P.O. Box 12087
La Jolla, CA 92039-2087
(858)450-5558
(800)854-3417
FAX: (858)453-3552

Catalog # 71079

In Case of Emergency Call Chemtrec®
(800)424-9300 (within U.S.A.)
(703)527-3887 (outside U.S.A.)

Product name RosettaBlue™ Competent Cell Set

2. Composition and Information on Ingredients

Ingredient Name	CAS No.	Product No.	EU Symbol	R-Phrases
Dimethyl Sulfoxide	67-68-5	RC1120	Xi	R36/38

Note: See section 8 for occupational exposure limits and section 11 for LC50/LD50 information.

3. Hazards Identification

Primary Hazards and Critical Effects : RC1120 CAUTION! MAY CAUSE EYE AND SKIN IRRITATION.
Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.

Physical/Chemical hazards : Not applicable.

Human Health Hazards : RC1120 Irritating to eyes and skin.

Environmental Hazards : Not applicable.

4. First Aid Measures

Inhalation : RC1120 If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Ingestion : RC1120 Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms appear.

Skin Contact : RC1120 In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Eye Contact : RC1120 In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Notes to Medical Doctor : Not available.

5. Fire-Fighting Measures

Extinguishing Media : Use foam or all purpose dry chemicals to extinguish.

Fire-Fighting Procedures : Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.

Fire/Explosion Hazards : Not applicable.

Hazardous Decomposition Products : These products are carbon oxides (CO, CO2), sulfur oxides (SO2, SO3...).

6. Accidental Release Measures

Personal Precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (Section 8). Follow all fire fighting procedures (Section 5).

Environmental Precautions and Clean-up Methods : If emergency personnel are unavailable, contain spilled material. For small spills add absorbent (soil may be used in the absence of other suitable materials) scoop up material and place in a sealed, liquid-proof container for disposal. For large spills dike spilled material or otherwise contain material to ensure runoff does not reach a waterway. Place spilled material in an appropriate container for disposal. Minimize contact of spilled material with soils to prevent runoff to surface waterways.

Note: See section 1 for emergency contact information and section 13 for waste disposal.

7. Handling and Storage

Handling	:	RC1120	Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
Storage	:	Keep container tightly closed. Keep container in a cool, well-ventilated area.	
Packaging Materials	:	Use original container.	

8. Exposure Controls and Personal Protection

Occupational Exposure Limits

Ingredient Name	Occupational Exposure Limits
RC1120	Not available.

Engineering Controls	:	RC1120	No special containment is required. Local exhaust ventilation should be provided.
Personal Protective Equipment			
Respiratory System	:	RC1120	Use appropriate respiratory protection if there is the potential to exceed the exposure limit(s).
Skin and Body	:	RC1120	Work uniform or laboratory coat.
Hands	:	RC1120	Use chemical resistant, impervious gloves. Additional body garments should be used based upon the task being performed (e.g., sleevelets, apron, gauntlets, disposable suits).
Eyes	:	RC1120	Safety glasses. Goggles, face shield, or other full-face protection if potential exists for direct exposure to aerosols or splashes.

9. Physical and Chemical Properties

Kit Components

69318: 1 x 2 ng Test Plasmid
69319: 4 x 2 ml SOC Medium
71034: 2 x 0.2 ml RosettaBlue™(DE3)pLysS Competent Cells, containing Dimethyl Sulfoxide (RC1120)
71058: 2 x 0.2 ml RosettaBlue™ Competent Cells, containing Dimethyl Sulfoxide (RC1120)
71059: 2 x 0.2 ml RosettaBlue™(DE3) Competent Cells, containing Dimethyl Sulfoxide (RC1120)

Flash Point

Not available.

10. Stability and Reactivity

Stability	:	RC1120	The product is stable.
Conditions and Materials to Avoid	:	RC1120	Reactive with oxidizing agents, reducing agents, acids.
Hazardous Decomposition Products	:		Not available.

11. Toxicological Information

Toxicity Data

Ingredient Name	Test	Result	Route	Species
RC1120	LD50	14500 mg/kg	Oral	Rat
	LD50	7920 mg/kg	Oral	Mouse
	LD50	>10000 mg/kg	Oral	Dog
	LD50	40000 mg/kg	Dermal	Rat
	LD50	50000 mg/kg	Dermal	Mouse
	LDLo	>11000 mg/kg	Oral	Guinea pig

Routes of Entry : Absorbed through skin. Dermal contact. Eye contact.

Acute Effects

Inhalation	:		Not available.
Ingestion	:	RC1120	Practically non-toxic if swallowed.
Skin Contact	:	RC1120	Moderately irritating to the skin. Practically non-toxic in contact with skin.
Eye Contact	:	RC1120	Moderately irritating to the eyes.

Chronic Effects

Adverse Effects	:	Not available.
Target Organs	:	Not available.
Carcinogenic Effects	:	Not available.
Mutagenic Effects	:	Not available.
Developmental and Teratogenic Effects	:	Not available.
Reproductive Effects	:	Not available.

Other Information : RC1120 Repeated or prolonged exposure is not known to aggravate medical condition.

12. Ecological Information

Ecotoxicity Data

Ingredient Name	Species	Period	Result
RC1120	Not available.	Not available.	Not available.

13. Disposal Consideration

Waste Handling and Disposal : Waste must be disposed of in accordance with federal, state and local environmental control regulations.

14. Transport Information

Air

IATA-DGR Class : Not controlled under IATA.

Packing Group

15. Regulatory Information

EU Regulations

Hazard Symbol(s)	:	Xi
Risk Phrases	:	R36/38- Irritating to eyes and skin.
Safety Phrases	:	S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

US Regulations

Haz-Com Standard	:	Not controlled under the HCS (United States).
EPA	:	Not available.
State	:	Not available.

Canadian Regulations

WHMIS	:	Not controlled under WHMIS (Canada).
CEPA	:	No products were found.
Provincial	:	No products were found.

16. Other Information

Validated by jcw on 11/11/2003.

Version : 1.0

Date of Printing : **11/14/2003.**

Notice to Reader

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Cell Biology

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Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing transfection host viruscide testing
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus

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Material Transfer Agreement New!
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- Cell, microbial and molecular genomics products for the life sciences

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

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

APPENDIX May 30th, 2012

MacDonald Lab, RRI, Room 7260C-1

Proposed experimental use of lentivirus

Primary cultured neurons are notoriously difficult to transfect by traditional means (e.g. lipofectamine, $CaPO_4$, etc). Moreover, although these approaches may be successfully utilized, the efficiency achieved (usually ~2-3%) is insufficient for biochemical studies and the reliability of the procedure is too variable. In contrast, we have routinely achieved >80% efficiency using lentivirus-mediated gene transduction. We will generate lentivirus, utilizing the two expression vectors listed in the modification form, for shRNA-mediated gene silencing (pLB vector) or for overexpression of genetically modified proteins (FUGW) in primary murine cultured neurons. Proteins targeted for silencing include EPAC (exchange protein directly activated by cAMP), DISC1 (disrupted-in-schizophrenia 1), TRPM2 (transient receptor potential, melastatin 2), NMDA receptor subunits (GluN1, GluN2A and GluN2B), and Pannexin and STIM channels (Panx1, Panx2, STIM1, and STIM2). For protein overexpression, HA- or FLAG-tagged TRPM2 will be expressed in our cultured neurons for immunostaining, immunoprecipitation and electrophysiological experiments.

Protocol for Handling Recombinant Replication-deficient Lentiviruses

Lentiviral vectors are different from the commonly used adenovirus based gene delivery systems because the gene of interest becomes stably integrated into the host cell's genome. The efficiency of lentiviral systems are due to the fact that they are actively imported into the nuclei of dividing, as well as non-dividing cells, as opposed to traditional retroviruses.

The lentiviral genome contains nine genes but only three of those are required to generate a replication-deficient virus. The three essential genes are Gag, Pol and Env and they can all be provided in trans. Gag encodes a capsid protein and Pol is required for the viral polymerase, RNase, protease and integrating functions. The Env, or, envelope gene encodes a transmembrane glycoprotein that also determines the tropism of the viral particle (ie. the specificity of the virus for a particular host cell). **In the ViraSafe Ecotropic Packaging system from Cell Biolabs, Inc. (which we will be using), the Env gene encodes a glycoprotein from Murine Leukemia Virus, thus providing a viral particle that can transduce only mouse and rat cells with high efficiency**. The remaining viral genome (*ie.* cis-elements only) are used to construct different Lentiviral cloning vectors and when the cloning vectors are transfected into packaging cell lines (usually 293 cells) also expressing the gag, pol and env protein in trans, replication-deficient Lentivirus particles can be generated that are carrying the gene of interest in the viral RNA genome.

Note! Only laboratory personnel that have been informed about safety precautions and working routines, and have permission from the person in charge are allowed to enter room 7260C-1 during Lentiviral work production. This also includes cleaners and service-personnel.

Principle:

All procedures for handling or manipulating Lentivirus should be carried out at Biosafety Level 2 (BL2) with the use of Containment Level 3 operational practices. All work will be done in a biological safety cabinet (BSC) by authorized personnel wearing coveralls, gloves, safety glasses and shoe covers (ie. full coverage protective clothing). Personal items (eg. purses) will not be brought into the containment room. All protective clothing will be removed upon completion of the work and left in the room or disposed of as waste (shoe covers, gloves). Protective items to be re-used will be autoclaved within room 7260C-1 using a portable autoclave. Coveralls will be kept on a coat rack within the containment room. No work with these viral vectors is permitted on the open bench.

Laboratory Facility:

The Principal Investigator has designated Room 7260C-1 for periodic lentiviral work, which contains a handwashing sink, biological safety cabinet (BSC), incubator, microscope, and CO2 source. This room is an inner lab with 2 doors between the BSC and the hallway and restricted entry to the lab. A sign stating that viral vectors are present, entry is restricted to authorized personnel, and doors are to remain closed will be posted on the laboratory door.

Working precautions for handling Lentivirus:

1. All experimental materials shall be handled with care.
2. The door to the containment room shall remain locked.
3. Within the BSC:
   a. For small quantities of low (cell lysate) and high (purified) titer Lentivirus, use sterile, aerosol barrier-containing pipette tips.
   b. For larger amounts (more than 1ml) of low titer lysates use sterile serological disposable pipettes.
   c. The maximum amount of infected growth media handled at one time should never exceed 500 ml.
4. Using a dunk tank, plastics will first be either filled (eg. pipet tips and serological pipettes) or rinsed (eg. plates and flasks) with Wescodyne Solution (20% Wescodyne/40% ethanol/40% water), drained, and then put into a high-density 4mil polyethylene plastic biohazard bag lined with a cardboard box prior to autoclaving.
5. Concentration of the viral particles will be done using Amicon Ultra-15ml 100k MWCO centrifugal filter devices. All centrifugation shall be done in closed buckets with aerosol-tight lids. Loading and unloading of samples into the sealed buckets will be done in the BSC. Buckets will be sprayed with 70% ethanol before removing from BSC.
6. Sharps shall be eliminated from experimental procedures to prevent injuries. No needles or Pasteur pipettes will be used in the production and use of lentivirus.
7. Gloves shall be worn at all times when working with viral vectors. Gloves will be sprayed with 70% ethanol and then removed by using the inside-out technique before disposing into biohazard waste to be autoclaved. Wash hands immediately after removing gloves and before

leaving work area. Never wear gloves outside of the laboratory, or touch things with gloved hands.

8. During any lentiviral work, signs and labels shall be placed to indicate each area where viral vectors are used and stored (BSC, incubators, freezer, laboratory entrance doors, etc.)

Decontamination and disposal procedures:

All materials that come in contact with viral particles must be properly decontaminated prior to disposal. Note that autoclaving of all materials will first be done within room 7260C-1 using a portable autoclave. Solid waste will then be autoclaved again through the central autoclave facility at Robarts.

1. ***Disposal/decontamination of solid waste such as, paper tissues, pipette tips, etc.***: All solid waste (including disposable plastic wares) should be discarded in biohazard bags for the appropriate treatment (autoclaving) according to institutional practices and guidelines prior to disposal.
2. ***Disposal/decontamination of liquid waste***: All liquid materials (Lentivirus-containing media, buffers, washes) should be decontaminated inside safety cabinet by addition of Sporgon Solution prior to autoclaving.
3. ***Work surfaces inside cabinets*** should be decontaminated with Sporgon Solution, followed by 70% ethanol.
4. ***Instruments, equipment*** and any other items that are not disposable and contact Lentivirus will be decontaminated with Sporgon Solution and/or autoclaved.
5. ***Routine laboratory cleaning*** will be done by lab personnel within the containment room.

Accidents:

Spills:

Effective disinfectants (10% bleach, Wescodyne or Sporgon Solution) will be made available in the laboratory at all times and for immediate use. In the event of a spill or container breakage resulting in the unintentional release of a biological agent:

(i) Place bleach soaked paper towel or absorbent on the liquid
(ii) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(iii) evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material, normally 20 minutes;
(iv) pour a strong disinfectant solution (i.e. 10% bleach) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(v) carefully place paper into a bag for incineration;
(vi) decontaminate all surfaces exposed to the spill with the disinfectant.

If aerosols may have been created in the spill or unintentional release, evacuate the laboratory for a time sufficient for most aerosols to settle, be dispersed, or removed by the ventilation system, usually 20-30 minutes. The use of respiratory protection should be considered for re-entry. Then proceed with items (i)-(v) above. During an emergency, the first priority is the protection of the health and safety of personnel, followed by the environment (i.e. sewer drains), followed by equipment or property.

Spills within a biological safety cabinet

- Leave the ventilation on
- All items within the cabinet should be disinfected (Walls and surfaces wiped down, equipment wiped down and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Ventilation should run 10-15 minutes before continuing work in BSC

Spills within an incubator

- All shelves and walls within the incubator should be disinfected (walls and surfaces wiped down, and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e. 10% bleach, Wescodyne or Sporgon Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes (close the door of the incubator during the disinfection time)
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Finish by wiping the incubator with 70% ethanol

Inhalation:

In case of inhalation, personnel should be directed to employee health for observation and maintained under medical surveillance. Cuts and abrasions should be treated as appropriate, according to their severity. Minor cuts should be treated with the Lab first Aid Kit (disinfectant wipe and band aid), otherwise personnel should be taken to emergency room for appropriate medical evaluation and care. Written records of all incidents should be maintained.

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Skin exposure:

Contaminated skin should be scrubbed with germicidal soap and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

lablife

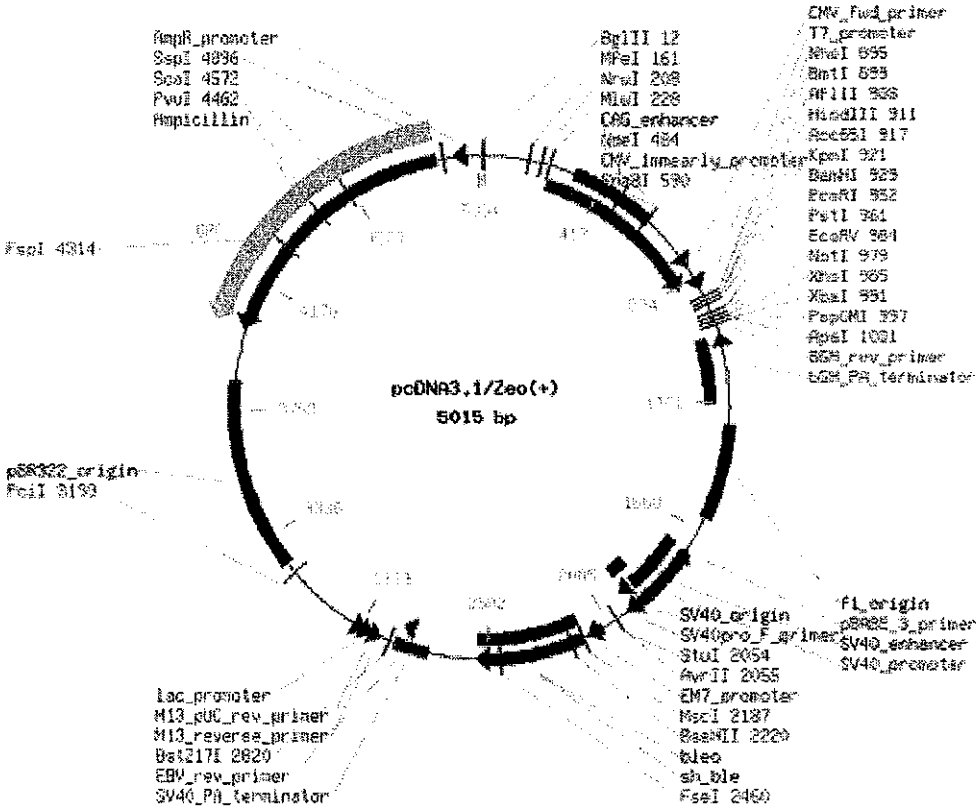
Community

Vector Database > pcDNA3.1/Zeo(+)

addgene **Vector Database**

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

Plasmid Name	pcDNA3.1/Zeo(+)
Source/Vendor	Invitrogen
Plasmid Type	Mammalian
Viral/Non-viral	Nonviral
Stable/Transient	Transient
Constitutive/Inducible	Constitutive
Promoter	CMV
Expression Level	High
Plasmid Size	5015
Sequencing Primer	T7 Fwd
Sequencing Primer Sequence	5'd[TAATACGACTCACTATAGGG]3'
Bacterial Resistance	Ampicillin
Mammalian Selection	Zeocin
Notes	Differs from other pcDNA3.1 in drug resistance; +/- refers to orientation of f1 ori.
Catalog Number	V86020
Link	http://www.invitrogen.com/content.cfm?pageid=8012&sku=V86020
Plasmid Sequence	View Sequence


pcDNA3.1/Zeo(+)
5015 bp
AmpR_promoter
SspI 4036
ScaI 4572
PvuI 4462
Ampicillin
FspI 4814
pBR322_origin
PciI 3139
lac_promoter
M13_pUC_rev_primer
M13_reverse_primer
Bst217I 2820
EBV_rev_primer
SV40_PA_terminator
BglII 12
MfeI 161
NruI 208
MluI 228
CAG_enhancer
KpnI 484
CMV_immearly_promoter
SnaBI 530
CMV_fwd_primer
T7_promoter
NheI 595
BmtI 633
AflII 936
HindIII 911
Asp651 917
KpnI 921
BamHI 925
EcoRI 952
PstI 961
EcoRV 984
NotI 979
XhoI 985
XbaI 991
PspOMI 997
AgeI 1001
BGH_rev_primer
bGH_PA_terminator
SV40_origin
SV40pro_F_primer
StuI 2054
AvrII 2055
EM7_promoter
NsiI 2187
BsaAI 2220
bleo
sh_ble
FseI 2460
f1_origin
pBR85_3_primer
SV40_enhancer
SV40_promoter

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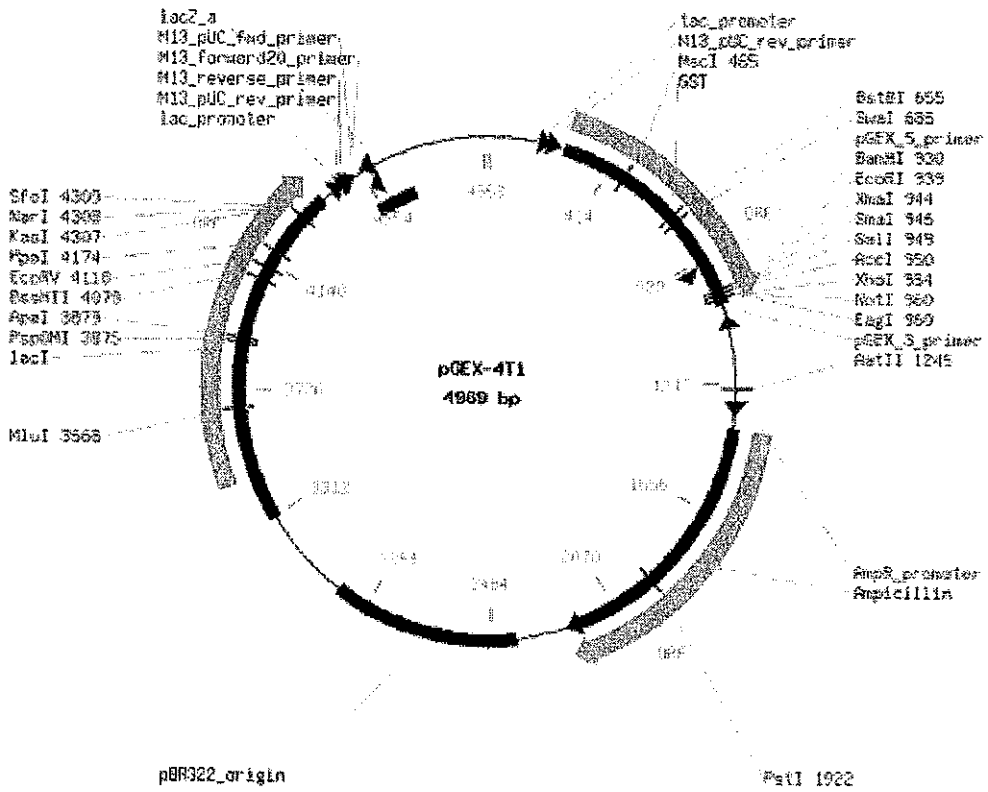
Community

Vector Database > pGEX-4T1

addgene **Vector Database**

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

Plasmid Name	pGEX-4T1
Alt Names	pGEX-4T-1
Source/Vendor	Amersham (now GE Healthcare)
Plasmid Type	Bacterial
Viral/Non-viral	Non-viral
Promoter	tac
Expression Level	High (activate with IPTG)
Plasmid Size	4969
Sequencing Primer	pGEX5'
Sequencing Primer Sequence	GGGCTGGCAAGCCACGTTTGGTG
Protein Tags	GST (Nterm)
Bacterial Resistance	Ampicillin
Notes	thrombin or factor Xa protease sites to cleave protein from fusion. pGEX-1lambdaT, pGEX-4T-1, pGEX-5X-1 accept cDNA from lambda gt11 libs. Hosts: E.coli. Related vectors: pGEX-2T. (Information source: VectorDB.)
Catalog Number	27458001
Link	http://seq.yeastgenome.org/vectordb/vector_descrip/PGEX4T1.html
Plasmid Sequence	View Sequence


lacZ_a
M13_pUC_fwd_primer
M13_forward20_primer
M13_reverse_primer
M13_pUC_rev_primer
lac_promoter
lac_promoter
M13_pUC_rev_primer
NcoI 465
GST
BstBI 655
SwaI 685
pGEX_5_primer
BamHI 920
EcoRI 939
XmaI 944
SmaI 945
SalI 949
AccI 950
XhoI 954
NotI 960
EagI 969
pGEX_3_primer
AatII 1245
SfoI 4303
NarI 4308
KasI 4307
MpeI 4174
EcoRV 4116
BsaAI 4078
ApaI 3879
PspOMI 3875
lacI
MluI 3568
pGEX-4T1
4969 bp
pBR322_origin
AmpR_promoter
Ampicillin
PstI 1922

pTNT™ Vector

The pTNT™ Vector is designed for the convenient in vitro expression of cloned genes. Both SP6 and T7 polymerase promoters lie in tandem adjacent to the multiple cloning site. This permits gene expression from either an SP6- or T7-based coupled in vitro transcription/translation system. The presence of RNA phage promoters also allows for the highly efficient synthesis of RNA in vitro. The pTNT™ Vector also contains a 5′ β-globin leader sequence and synthetic poly(A)$_{30}$ tail, both of which have been shown to enhance expression of certain genes.

...

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Figures

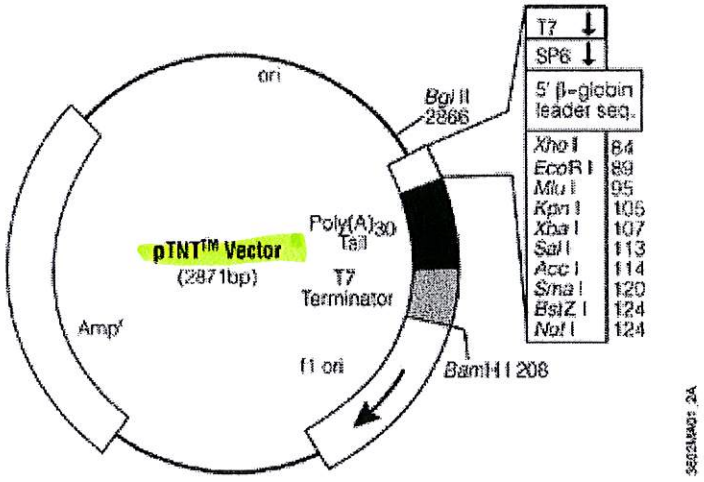
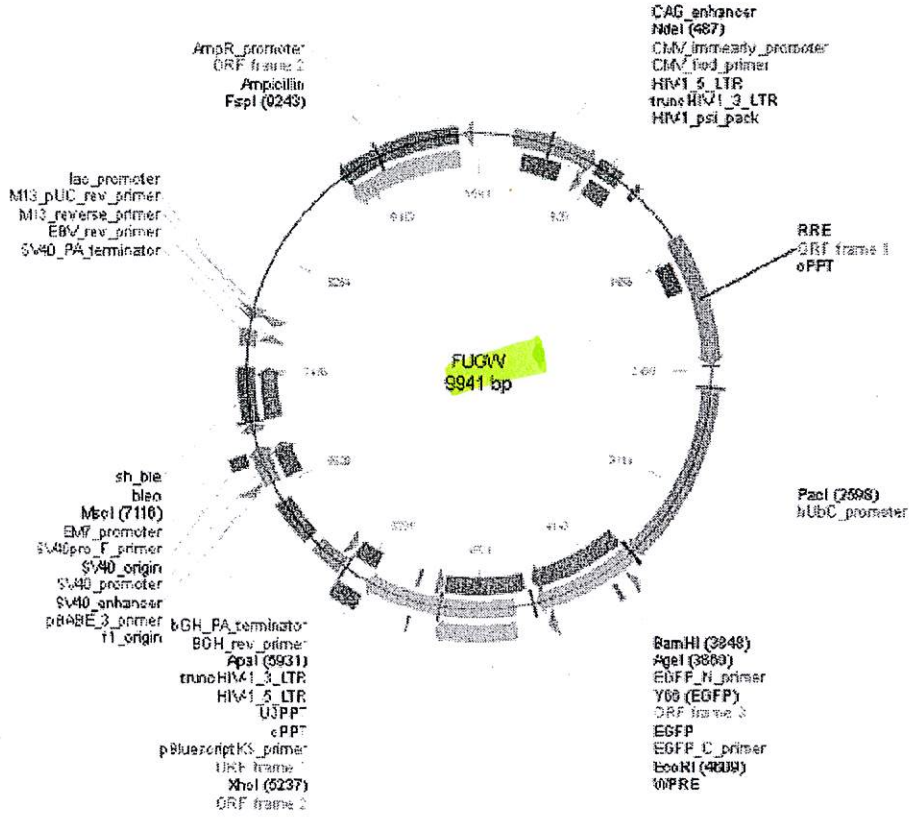


Figure 1. pTNT™ Vector.

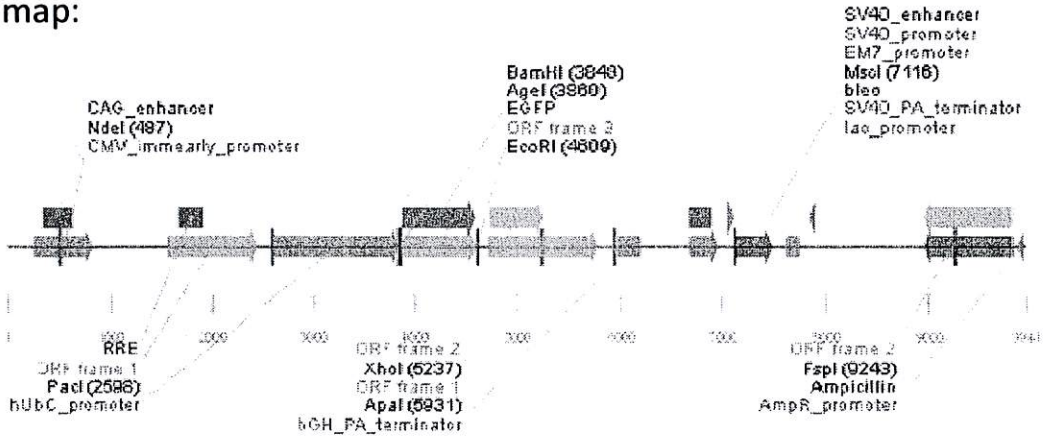
Plasmid pFUGW is a transfer vector that will be used to express full length proteins after transduction within primary cultured neurons (mouse or rat). pFUGW will be packaged within replication deficient lentivirus following co-transfection of HEK 293 cells with packaging vectors (3rd generation) already listed on our biohazard permit (pRSV, pCgpV and pCMV(Eco)).

pFUGW map:



FUGW: 9941 bp

linear map:



addgene
A better way to share plasmids

Browse > Stephan Kissler > Kissler et al > pLB

Plasmid 11619: pLB

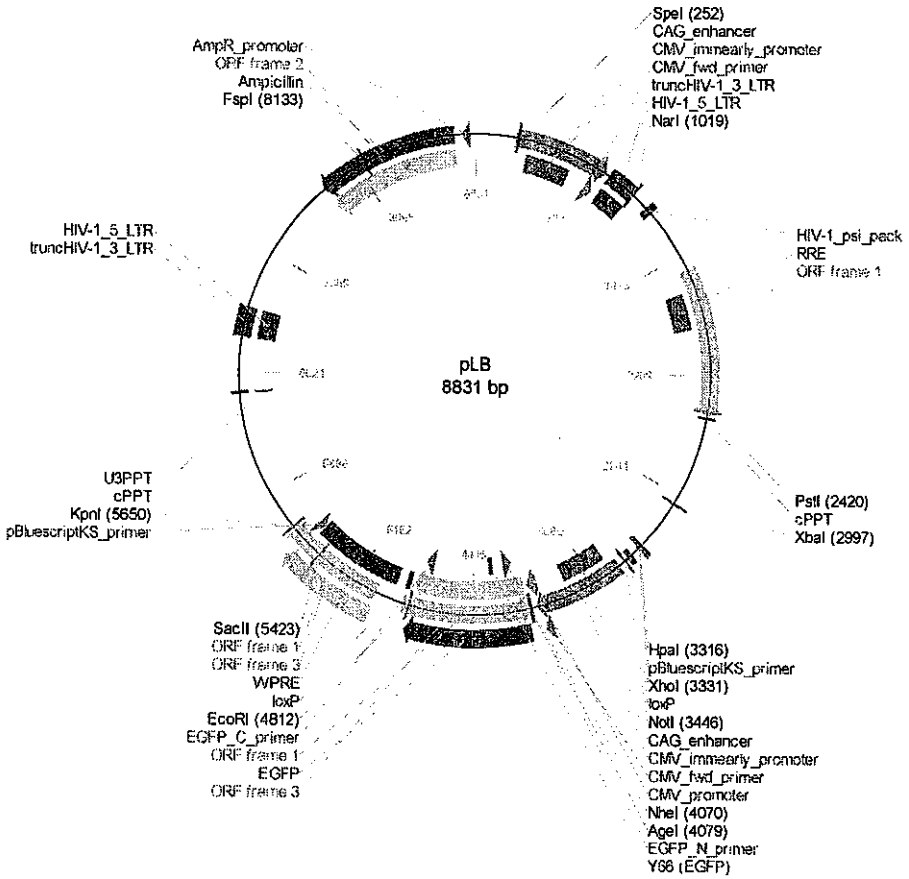
Gene/insert name:	None
Fusion protein or tag:	GFP
Terminal:	C terminal on backbone
Vector backbone:	pLL3.7 (Search Vector Database)
Backbone manufacturer:	N/A
Vector type:	Mammalian Expression, Lentiviral, RNAi, Cre/Lox
Backbone size w/o insert:	8500
Cloning site 5':	HpaI
Site destroyed during cloning:	No
Cloning site 3':	XhoI
Site destroyed during cloning:	No
5' sequencing primer:	mU6-F List of Sequencing Primers
Bacterial resistance:	Ampicillin
Growth strain:	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Sequence:	View sequences (2)
Map:	View map
Principal Investigator:	Stephan Kissler
Terms and Licenses:	MTA

Comments: pLB is a modification of pLL3.7. Two genetic elements known to prevent epigenetic silencing were added. A fragment of one antirepressor element (#40) was cloned upstream of the U6 promoter and a scaffold-attached region (SAR) was cloned downstream of GFP.

Please see author's map for more detailed information.

Note: A single base pair deletion at position 11 of the U6 promoter in this plasmid does not impair the efficacy of this reagent. There is also a base pair insertion upstream of the promoter. The depositing lab has no indication that it functionally impairs pLB.

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Feature Name	Start	End
CMV_immearly_promoter	239	815
CAG_enhancer	318	605
CMV_fwd_primer	772	792
truncHIV-1_3_LTR	835	1015
HIV-1_5_LTR	835	1015
HIV-1_psi_pack	1126	1170
RRE	1686	1919
cPPT	2450	2465
pBluescriptKS_primer	3332	3348
loxP	3391	3424
CMV_immearly_promoter	3489	4041
CAG_enhancer	3544	3831
CMV_fwd_primer	3998	4018
CMV_promoter	3999	4068
EGFP	4095	4808
EGFP_N_primer	4158	4137
Y66 (EGFP)	4275	4304
EGFP_C_primer	4745	4766
loxP	4831	4864
WPRE	4922	5509
pBluescriptKS_primer	5528	5512
cPPT	6500	6515
U3PPT	6500	6521
truncHIV-1_3_LTR	6837	7017
HIV-1_5_LTR	6837	7017
Ampicillin	8698	7838
AmpR_promoter	8768	8740

ORF	Start	End
ORF frame 1	1564	2451
ORF frame 1	4847	4077
ORF frame 3	4092	4811
ORF frame 3	5010	5564
ORF frame 1	5023	5664
ORF frame 2	8698	7838

Enzyme Name	Cut
SpeI	252
NarI	1019
PstI	2420
XbaI	2997
HpaI	3316
XhoI	3331
NotI	3446
NheI	4070
AgeI	4079
EcoRI	4812
SacII	5423
KpnI	5650
FspI	8133

Article: In vivo RNA interference demonstrates a role for Nramp1 in modifying susceptibility to type 1 diabetes. Kissler et al (Nat Genet. 2006 Apr . 38(4):479-83. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 11619" in your Materials and Methods section.

Product Manual

ViraSafe™ Lentiviral Packaging System, Ecotropic

Catalog Number

VPK-205	1 kit

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells. Lentivirus pseudotyped with the MLV ecotropic envelope glycoprotein will only transduce mouse and rat cells with high efficiency. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion (Figure 1). Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, these systems still present a small chance of generating replication-competent lentivirus upon recombination. In addition, most commercial lentiviral packaging systems provide plasmids containing the viral structure proteins in a premixed formulation, making it nearly impossible to optimize the ratio of the various plasmids for your particular experiment and host cell.

Cell Biolabs' ViraSafe™ Lentiviral Packaging System provides a much safer method to package lentivirus, while still providing high viral titers. In addition, each plasmid is provided separately rather than in a packaging mixture. This allows you the flexibility to amplify individual plasmids and optimize the ratio of plasmids for your experiment.

Key Features of ViraSafe™ Lentiviral Packaging System:

1. Packaging Plasmids: Improve the packaging plasmid to increase performance and reduce the likelihood of recombination between vector components.
   a. Minimize HIV sequences – no accessory proteins, Tat or Rev, or LTRs
   b. Prevent overlap with vector SM by codon wobbling Gag sequences
   c. Boost particle production by incorporating adenovirus VA_I element
2. Flexible: All vectors including packaging vectors are provided separately to allow end-user to optimize the vector ratio for maximal lentivirus production.

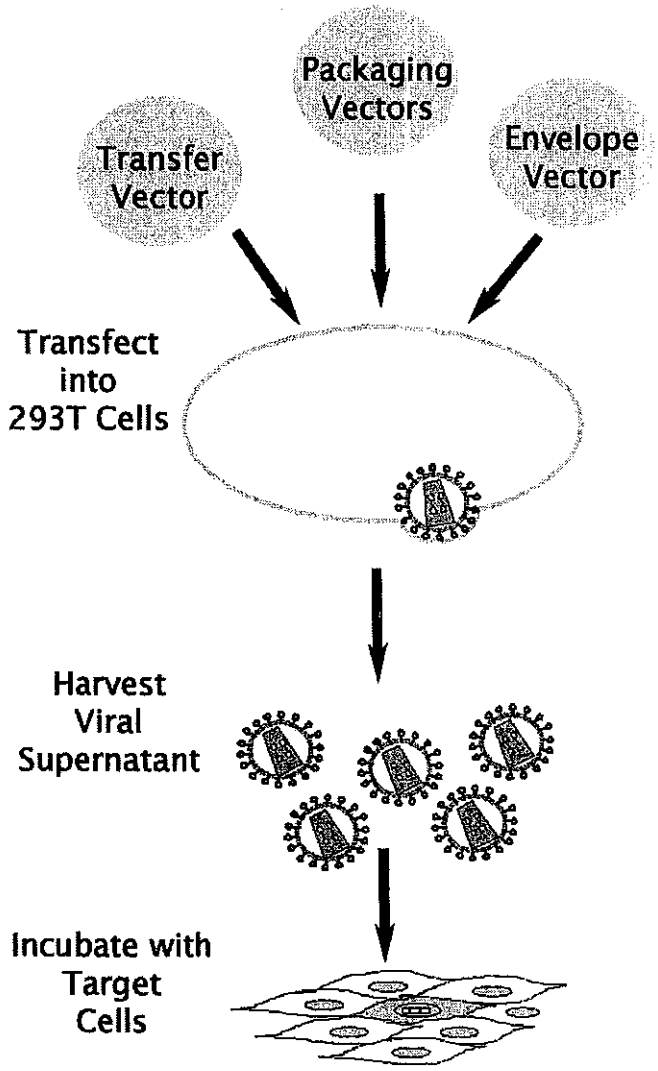


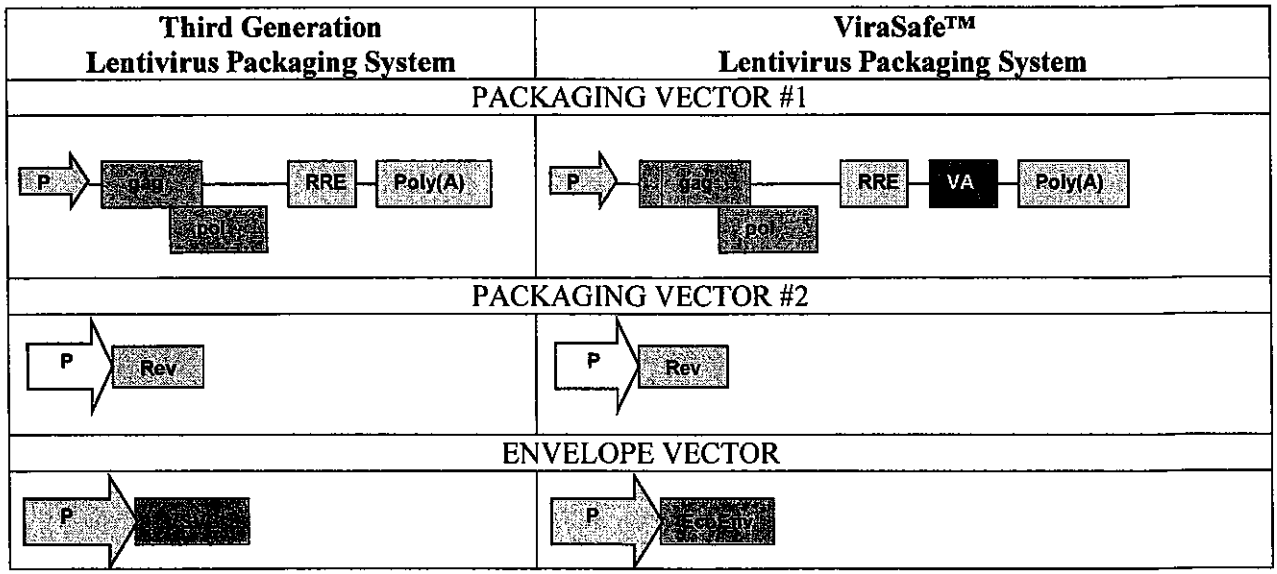
Figure 1. Lentivirus Production in 293T Cells

Related Products

1. LTV-100: 293LTV Cell Line
2. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
3. LTV-300: GFP Lentivirus Control
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. VPK-104: ViraBind™ Lentivirus Purification Kit
6. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
7. VPK-108-F: QuickTiter™ Lentivirus Quantitation Kit (FIV p24 ELISA)

8. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
9. VPK-200: ViraSafe™ Universal Lentivirus Expression System
10. VPK-206: ViraSafe™ Lentivirus Packaging System, Pantropic
11. VPK-211: pSMPUW Universal Lentiviral Expression Vector (Promoterless)
12. VPK-220: pSMPUW miR-GFP/Puro Lentiviral Expression Vector

Unique Elements of the ViraSafe™ Lentivirus Packaging System



Vector Name	Element	Name	Benefits compared to 3rd Generation System
ELEMENTS ADDED			
Packaging Vector #1		Codon Wobble	• Increased safety: reduces sequence homology
	VA	Adenovirus VA	• Increased viral titer

Kit Components

1. pRSV-Rev Packaging Vector (Part No. 320022): One 40 µL vial at 0.25 mg/mL.
2. pCMV-Eco Envelope Vector (Part No. 320026): One 40 µL vial at 0.25 mg/mL.
3. pCgpV Packaging Vector (Part No. 320024): One 40 µL vial at 0.25 mg/mL.

Figure 2: pRSV-Rev Packaging Vector (4180 bp, **Ampicillin**-resistant). EcoRI Digestion: 300 bp + 3880 bp

Figure 3: pCMV-Eco Envelop Vector (6763 bp, **Ampicillin**-resistant). BamHI Digestion: 777 bp + 5986 bp.

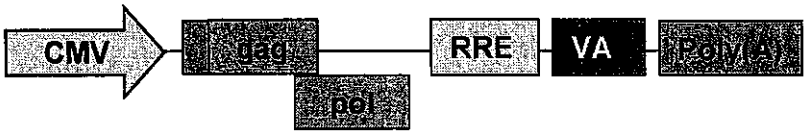


Figure 4: pCgpV Packaging Vector (9118 bp, **Ampicillin**-resistant). Pst I Digestion: 927 bp + 1424 bp + 6767 bp.

Materials Not Supplied

1. Lentiviral Transfer Vector
2. 293T cells: we recommend 293LTV Cell Line (Cat. # LTV-100) for high titer production of lentivirus.
3. Cell Culture Medium
4. Transfection Reagents

Storage

Upon receipt, store all other kit components at -20°C until their expiration dates.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ViraSafe™ Universal Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

Lentivirus Production

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (cat.# LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

   Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (transfer vector: pCMV-Eco:pRSV-REV:pCgpV).

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 μm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

Post-Packaging Considerations

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

1. **Concentration and purification of your lentivirus**: Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).

2. **Measure the titer of your lentivirus**: This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.

3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

References

1. Chen, M. et al. (2002). *Nature Genetics* **32(4)**: 670-675.
2. Naldini, L., U. Blomer, P. Gallay, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* **272**:263-267.
3. Verma, I. M., and N. Somia (1997) *Nature* **389**:239-242

4. Kahl C. A., Marsh J., Fyffe J., Sanders D. A., and K. Cornetta (2004) *J Virol.* **78**:1421-30.
5. White S. M., Renda M., Nam N. Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B. J., Federoff H., Pandya S., Rosenblatt J. D., and V. Planelles (1999) *J Virol.* **73**:2832-40.
6. Kafri T., van Praag H., Ouyang L., Gage F. H., and I. M. Verma (1999) *J Virol.* **73**:576-84.

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CELL BIOLABS, INC.
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Material Safety Data Sheet

SECTION 1. PRODUCT IDENTIFICATION

Catalog Number: VPK-200 through VPK-230
Product Name: ViraSafe™ Lentiviral Expression Systems

MANUFACTURER:

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126

EMERGENCY CONTACT:

+1 858 271 6500
info@cellbiolabs.com

SECTION 2. COMPOSITION/INFORMATION ON INGREDIENTS

Plasmid DNA in TE Buffers

SECTION 3. WASTE DISPOSAL

For small quantities: Cautiously add to a large stirred excess of water. Adjust the pH to neutral. Flush the aqueous solutions down the drain with plenty of water.

SECTION 4. FIRST-AID MEASURES

- IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
- IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN.
- IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN.

SECTION 5. SAFETY HANDLING PROCEDURES

- Should be handled by trained personnel observing good laboratory practices.
- Avoid breathing vapor.
- Avoid skin contact or swallowing.
- May cause allergic reaction in sensitized individuals.

SECTION 6. ACCIDENTAL RELEASE MEASURES

EVACUATE AREA. WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES. ABSORB WITH SAND OR VERMICULITE, SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL. AVOID RAISING DUST. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

The above information is believed to be correct but does not purport to be all inclusive and should be used only as a guide for experienced personnel. Cell Biolabs, Inc. shall not be held liable for any damage resulting from the handling or from contact with the above product(s).

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email: techsupport@alomone.com http://www.alomone.com

Molecular Tools for the Life Science Community

Certificate of Analysis

MATERIAL SAFETY DATA SHEET

Section 1 - Product Information

Product Name: Tetrodotoxin (with citrate), or (citrate free).
Cat. #: T-550/T-500.
Molecular Formula: $C_{11}H_{17}N_3O_8$.
Molecular Weight: 319.28 daltons.
CAS No.: 4368-28-9.
Chemical Name: Octahydro-12-(hydroxymethyl)-2-5,9;7,10a-dimethanol-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol.

Section 2 - Physical and Chemical Characteristics

Appearance: Colorless Solid.
Solubility: Soluble in acidic buffer (pH 4.8) or Methanol.

Section 3 - Physical Hazards

Flash Point: Not determined.
Classification: Not determined.
Extinguishing Fire: Use carbon dioxide, dry chemical extinguishers or water. An approved self-contained breathing apparatus and protective clothing are recommended.

Section 4 - Reactivity Data and Storage Conditions

Stability: Stable. Not a significant hazard in milligram quantities.
Storage Conditions: Freezer storage recommended.

Section 5 - Health Hazard Information

Routes of Entry: May enter the body through inhalation, ingestion, and eye and skin contact.
RTECS No.: IO1450000
Exposure limits: Not determined.
Toxicity: LD50; 334 µg/kg, oral, mouse; LD50: 7.3 µg/kg, intravenous, mouse.
Health Hazards: See Toxicity above and Potential Hazards.
Potential Hazards: Highly Toxic.
Carcinogenicity: Not listed by NTP, IARC or OSHA.
Exposure Symptoms: Unknown. Handle with care.
First Aid: Potentially harmful; avoid prolonged or repeated exposure. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with water for 15 minutes and seek medical advice. If inhaled, move individual to fresh air and seek medical advice. If swallowed, seek medical advice.

Section 6 - Precautions for Safe Handling, Use and Control Measures

Ventilation: Mechanical and respiratory protection are recommended.
Handling: Gloves, protective clothing and eyewear should be worn and safe laboratory practices followed.

In Case of Spill: Use appropriate protective equipment and methods to clean up spilled substance promptly. Absorb spill onto an appropriate material. Collect and dispose of all waste in accordance with applicable laws.
Clean up: Wash with soap and water.

Disclaimer: The information provided is believed to be correct to our best knowledge, but does not purport to be all inclusive, and shall be used only as a guide. This material is sold for research purposes only and is not required to appear on the TSCA inventory. It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual, experienced in handling potentially hazardous chemicals. Alomone Labs shall not be held liable for any damage resulting from handling or contact with the above product.

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Last Update: February, 2010.

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email: techsupport@alomone.com http://www.alomone.com

Molecular Tools for the Life Science Community

Certificate of Analysis

Tetrodotoxin (with citrate)

(TTX)

Cat. #: T-550

Origin: *Tetraodon pardalis* (puffer fish).

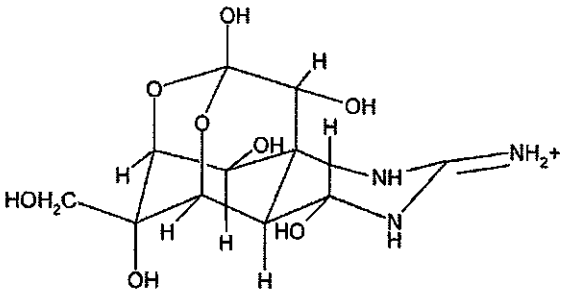
Source description: Natural.

M.W.: 319.28 daltons.

Purity: >98%.

Effective concentration: 100 nM - 1µM.

Structure:



Chemical name: Octahydro-12-(hydroxymethyl)-2-imino-5,9:7,10a-dimethan o-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol.

Molecular formula: $C_{11}H_{17}N_3O_8$.

CAS No.: 4368-28-9.

Activity: Tetrodotoxin is a potent and selective blocker of a subclass of Na_v channels[1], and is often used to define subclasses of Na_v channels[2].

References:

1. Narahashi, T. *et al.* (1964) *J. Gen. Physiol.* **47**, 965.
2. Hille, B. (2001) *Ion Channels in Excitable Membranes* (Third Edition) Chapter 3.

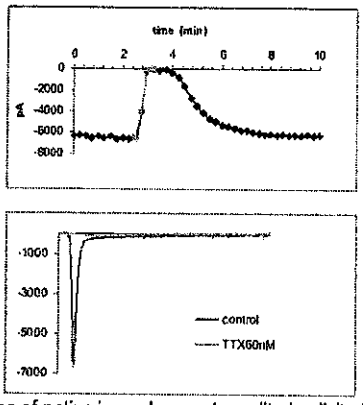
Sizes: 1 mg or 5 x 1 mg lyophilized powder.

Storage before reconstitution: Lyophilized powder can be stored intact at room temperature for several weeks. For longer periods, it should be stored at 4°C.

Reconstitution: Water. Centrifuge all product preparations before use (10000 x g 5 min).

Storage and stability after reconstitution: Up to one week at 4°C or six months at -20°C.

Bioassay: Tetrodotoxin (with citrate) inhibits native Na_v currents in ND7-23 cells.



Top: Time course of native inward current amplitude elicited from a holding potential of -100 mV by a 40 ms test pulse to -10 mV delivered every 15 sec. Application of 60 nM **Tetrodotoxin (with citrate)** (#T-550) is marked in cyan blue. Bottom: Superimposed current traces before (blue) and during (cyan) bath perfusion of 60 nM Tetrodotoxin (with citrate).

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TOXIN USE RISK ASSESSMENT

Name of Toxin:	Tetrodotoxin
Proposed Use Dose:	**1000** µg
Proposed Storage Dose:	**5000** µg
LD $_{50}$ (species):	10 µg

Calculation:

10 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 500

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

Comments/Recommendations: