Modification Form for Permit BIO-RRI-0031

Permit Holder: Robert Gros

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

Bonan Liu

Jozef Chorazyczewski

Qingming Ding

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E.coli (DH5 alpha), Adenovirus	
Approved Primary and Established Cells	Human (established) blood, Rodent (established) vascular. Human (primary) HEK 293, Rodent (primary) Rat/mouse vasc. SMCs	
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid: volunteers/patients	Human adipose (fat) tissue biopsy: patients
Approved Genetic Modifications (Plasmids/Vectors)	[bacteria]E. coli DH5alpha [plasmids] PDC316, PDC316. [virus] adenovirus [vector] Adeno-GFP, -MR-GPR30, - GRK2 - Acs	
Approved Use of Animals		
Approved Biological Toxin(s)		

* *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 STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: Jul 9, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): Ronald Westerby Nov. 25, 2010

Chair, Biohazards Subcommittee: _____ **Date:** _____

Description of Research Projects.

In our laboratory we utilize a variety of isolated tissues/cells, including primary and established cultures of rodent (rat and mouse) vascular smooth muscle cells, whole blood isolated from research volunteers and/or patients as well as adipose (fat) tissue biopsy samples from patients to examine the following:

a) The effect(s) of high blood pressure on cellular function is isolated cells from rodents and/or humans.
b) Regulation of isolated cells (rat, mouse or human) in response to steroid hormones
c) Alterations in the expression patterns of hormone receptors in isolated cells/tissues obtained from rat, mouse or human.

For many of these experiments we require adenoviral constructs in order to regulate the expression of various proteins/genes of interest in these isolated cells.

All of the adenoviral constructs listed in the Biohazardous Agents Registry Form have been generated using the Microbix adenovirus vector creation kit as previously described in some of our publications (Ding et al., 2009, Gros, et al., 2006; Gros, et al., 2007).

Briefly, rat or mouse vascular smooth muscle cells will be isolated via enzymatic digestion as previously described in detail (Gros, et al, 2006; Ding, et al., 2009; Gros, et al., 2007) and maintained in culture for various experiments and assays. Of note, live animals are only to be used a source of tissues and cells and will not be used for infection with the various adeno-viral constructs. For isolated human mononuclear leukocytes we utilize Ficoll-Hypaque separation protocol as previously described in detail (Gros, et al., 2007

Cells maintained in culture are infected using the various adenoviral constructs. Briefly, cells are incubated with adenoviral constructs for 12-16h following which cells are washed and culture media replaced. Forty eight hours post-infection cells are utilized for various assays including arborization, contraction as well as western blotting.

Please refer to the standard operating procedure listed below:

HUMAN ADIPOSE TISSUE EXPERIMENTS --- Standard Operating Procedures:

- Laboratory coats, gloves and safety glasses are worn while handling human adipose tissue. Universal level 2 precautions will be observed.

- Human adipose tissues are handled inside the biological safety cabinet (BSC) in room 4244C.

- Isolation of adipocytes for proteins, DNA or RNA are performed via enzymatic digestion in sealed 50 mL conical tubes. All centrifugation steps are done in sealed conical tubes.

- All serological pipettes, pipette tips are decontaminated in a solution of clidox, quatricide or diluted bleach (1:10 dilution of household bleach) for 30 minutes prior to discarding into biohazard waste container.

- Upon completion of digestion/isolation, all work surfaces and equipment used during the handling of human blood products are sprayed with clidox, quatricide or diluted bleach solution followed by a wash with 70% ethanol and air-dried.

- All solid waste materials related to the adenoviral experiments are placed in biohazard waste bag and sealed for disposal (i.e. to be autoclaved).

- Vacuum lines are HEPA filtered prior to entering into the vacuum system. For aspirated liquid waste, aspirate full-strength bleach through the suction tube into the liquid waste container to the approximate final concentration (1 in 10) and soak for 15 minutes and empty entire contents down the drain. Rinse drain and liquid waste flask with 70% ethanol.

- The resultant protein, DNA or RNA samples will be stored at -80 for subsequent use.

Product Data Sheet

pSMPUW Universal Lentiviral Expression Vector (Promoterless)

CATALOG NUMBER: VPK-211 **STORAGE:** -20°C

QUANTITY AND CONCENTRATION: 10 µg at 0.25 µg/µL in TE

Background

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. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. 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*.

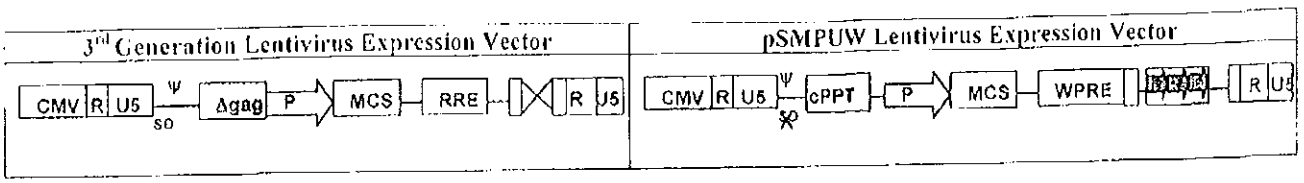
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, 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.

pSMPUW Universal Lentiviral Expression Vector (Promoterless) does not contain any promoter ahead of the multiple cloning sites, nor does it contain any reporter genes or antibiotic selection markers. This makes the system truly universal by allowing you to introduce your own promoter, marker or reporter that is optimal for your gene of interest or target cell. It also makes the system ideal for promoter studies. The expression vector can accommodate inserts up to 10 kb.

Related Products

1. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
2. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

Unique Elements of the pSMPUW Universal Lentivirus Expression Vector

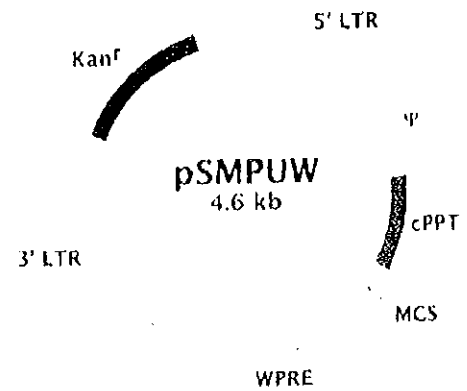


Element	Name	Benefits compared to 3rd Generation System
	ELEMENTS ADDED	
cPPT	Central Polypurine Tract	• Increased gene expression levels
	Hybrid 3' LTR Poly(A)	• Increased safety: prevents read-through transcription • Increased viral titer: vector transcript more stable in packaging cells
WPRE	WPRE	• Increased viral titer
	ELEMENTS DELETED	
Δgag	Gag sequence	• Increased safety: reduces sequence homology
RRE	Rev-Responsive Element	• Increased safety: reduces sequence homology

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.

pSMPUW Vector



MCS: GGGGGATCCGCGGAATTCGTCGATATCAGCGTCGACAAT
BamHI EcoRI EcoRV SalI

Figure 1: pSMPUW Lentiviral Expression Vector (4632 bp, **Kanamycin**-resistant). *Note: Bacterial culture of pSMPUW vector should be done in medium containing* ***10 µg/mL*** *Kanamycin.*
EcoRI/XhoI Digestion: 1251 bp + 3381 bp

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 (pSMPUW: pCMV-VSV-G: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). Add Millipore filter

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.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by a license from CHLA and University of Southern California. By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS 's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

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Worldwide: +1 858-271-6500
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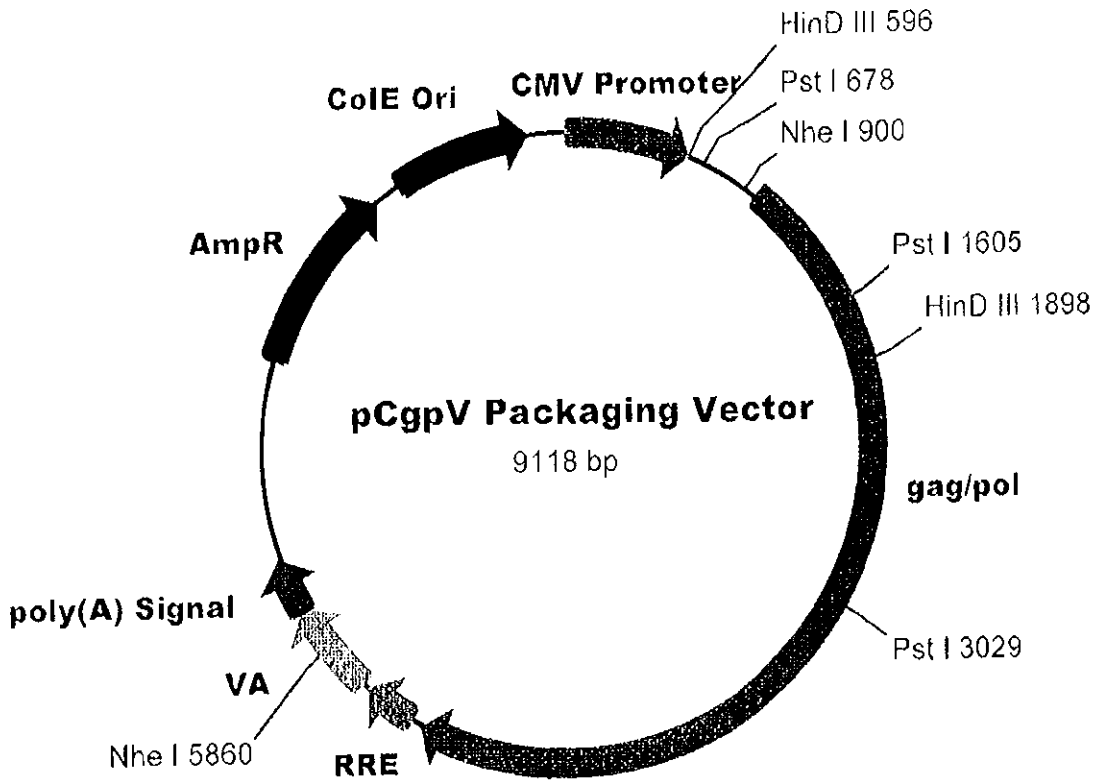
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com



Comments for pCgpV packaging vector

1-589bp: CMV Promoter

975-5281bp: HIV gag/pol sequence

975-2477bp: gag coding sequence
2270bp: gag/pol frameshift
2270-5281bp: pol coding sequence

5346-5578bp: HIV Rev response element (RRE)

5633-6065bp: Adenovirus VA RNA sequence

6066-6315bp: SV40 late polyadenylation signal

7273-8133bp: Amp resistance gene

8280-8919bp: ColE ori

Fragments created by PstI digest: 927bp+1424bp+6767bp=9118bp

Fragments created by NheI digest: 4158bp+4960bp=9118bp

Fragments created by Hind III digest: 1302bp+7816bp=9118bp

Plasmid 11619: pLB

Gene/insert name:	None
Insert size (bp):	Unknown
Fusion proteins or tags:	GFP
Terminal:	C terminal on backbone
Vector backbone:	pLL3.7 (Search Vector Database)
Backbone manufacturer:	N/A
Type of vector:	Mammalian expression,Lentiviral,RNAi,Cre/Lox
Backbone size (bp):	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)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	View sequence
Author's Map:	View map
Plasmid Provided In:	DH5a
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.

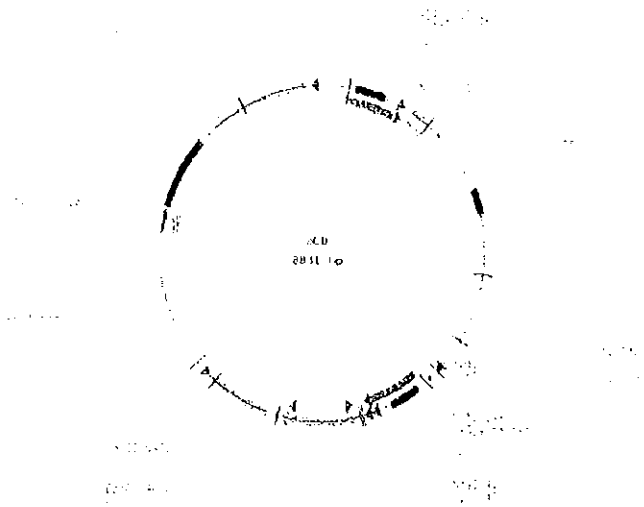
Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge

Plasmid Links
Author's map
Sequence
Related Plasmids
From this article
Stephan Kissler Lab Plasmids
Mammalian RNAi Tools

This is commonly requested with
pLKO.1 - TRC cloning vector
psPAX2
pLKO.1 - TRC control
pLL3.7
pMD2.G

Recently Viewed
pLB
Plasmid 11619



Selected features	
CAG_enhancer	318 - 605
CMV_immearly_promoter	239 - 815
CMV_fwd_primer	772 - 792
HIV-1_5_LTR	835 - 1015
truncHIV-1_3_LTR	835 - 1015
HIV-1_psi_pack	1126 - 1170
RRE	1686 - 1919
Orf frame 1	1564 - 2451
cPPT	2450 - 2465
pBluescriptKS_primer	3332 - 3348
loxP	3391 - 3424
CAG_enhancer	3544 - 3831
CMV_immearly_promoter	3489 - 4041
CMV_fwd_primer	3998 - 4018
CMV_promoter	3999 - 4068
EGFP_N_primer	4158 - 4137
EGFP	4092 - 4808

Unique restriction sites	
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

Orf frame 3	4092 - 4811
Orf frame 3	4847 - 4077
EGFP_C_primer	4745 - 4766
loxP	4831 - 4864
WPRE	4922 - 5509
Orf frame 1	5023 - 5664
pBluescriptKS_primer	5528 - 5512
cPPT	6500 - 6515
U3PPT	6500 - 6521
HIV-1_5_LTR	6837 - 7017
truncHIV-1_3_LTR	6837 - 7017
pBR322_origin	7683 - 7064
Orf frame 2	8698 - 7838
Ampicillin	8698 - 7838
AmpR_promoter	8768 - 8740

Article: In vivo RNA interference demonstrates a role for Nramp1 in modifying susceptibility to type 1 diabetes. Kissler S 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. This information allows Addgene to create a link from the plasmid page to your publication.

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.

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.

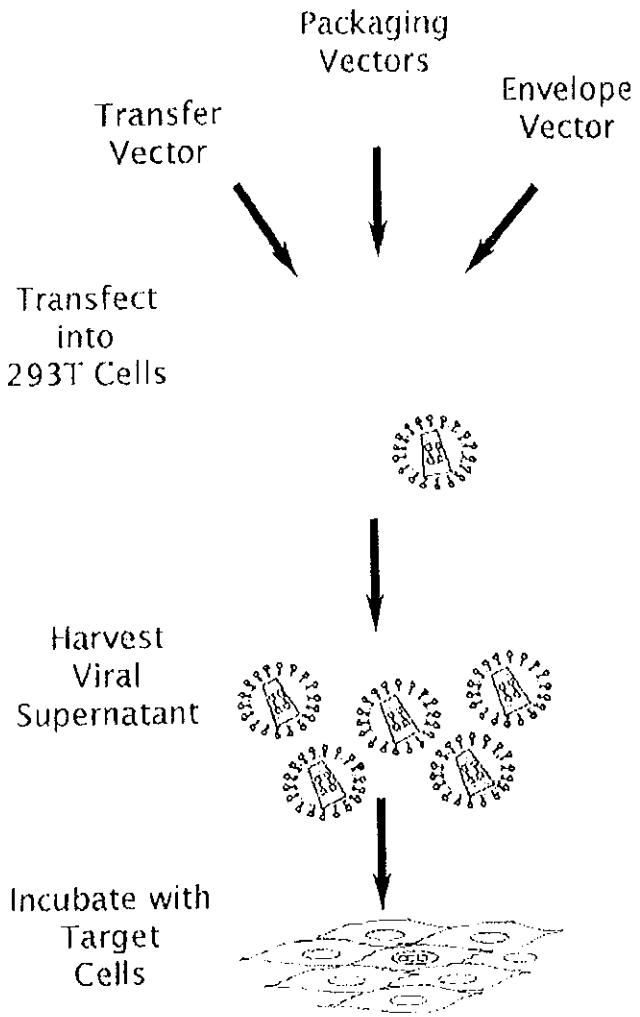
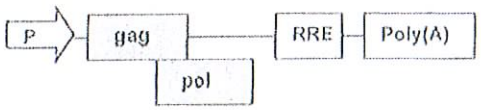
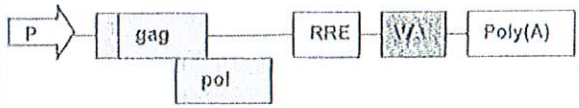
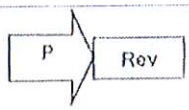
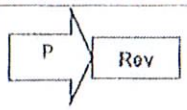
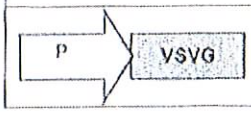
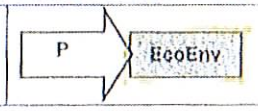


Figure 1. Lentivirus Production in 293T Cells

Related Products

1. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
2. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
3. VPK-108: QuickTiter™ Lentivirus Quantitation Kit
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
6. LTV-100: 293LTV Cell Line

Unique Elements of the ViraSafe™ Lentivirus Packaging System

Third Generation Lentivirus Packaging System	ViraSafe™ Lentivirus Packaging System
PACKAGING VECTOR #1	
	
PACKAGING VECTOR #2	
	
ENVELOPE VECTOR	
	

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

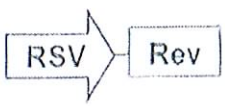


Figure 2. pRSV-Rev Packaging Vector (4180 bp, **Ampicillin**-resistant). EcoRI Digestion: 300 bp + 3880 bp

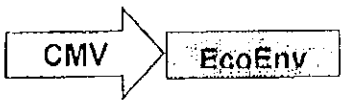


Figure 3: pCMV-Eco Envelop Vector (6763 bp, **Ampicillin**-resistant). BamHI Digestion: 777 bp + 5986 bp.

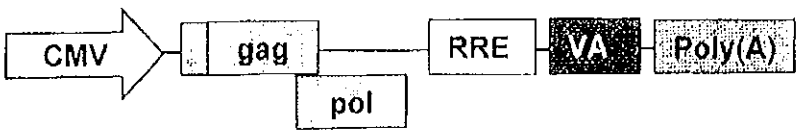


Figure 4: pCgpV Packaging Vector (9118 bp, **Ampicillin**-resistant). Pst I Digestion: 927 bp + 1424 bp + 6767 bp.

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. Kafri C. A., Marsh J., Eyffe J., Sanders D. A., and K. Cornetta (2003) *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.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by a license from University of Southern California. By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

Subject: Fw: Lentivirus use
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Tue, 06 Apr 2010 09:33:02 -0400
To: jstanle2@uwo.ca

FYI, Ron

----- Original Message -----
From: Mike Jackson <mjackson@robarts.ca>
To: Ron Noseworthy
Sent: Tue Apr 06 09:28:49 2010
Subject: Lentivirus use

Hi Ron,

Just wished to clarify that we will not be using lentivirus for any live animal work. I had forgotten to add a statement to that effect in our biohazard modification form.

Thanks, Mike

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 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 are 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. 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 required 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 (Stevenson-Lawson Building, Room 295) 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 John MacDonald / Michael Jackson
SIGNATURE
DEPARTMENT Robarts Research Institute/ Physiol & Pharm
ADDRESS 100 Perth Drive
PHONE NUMBER 33850
EMAIL jmacd53@uwo.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 1260C 1.9C level 2

*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: CIHR, HSFC, Canadian Stroke Network
GRANT TITLE(S): 1)Cell Signalling, NMDA Receptors and Hippocampal Metaplasticity (MOP-11C03) 2)The role of TRP channels in stroke, aging and calcium sensing in the central nervous system (MOP-15514) and 3)Neuroprotection: Preventing cell death and neuronal damage from stroke

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:

Natalie Lavine
Lidia Brandes
Michelle Olah
Kai Yang
Bikram Sidhu
Xiaowei Chen
Hongbin Li
Qies Hussein
Michael Jackson

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

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)? ⊗ YES O NO
If no, please proceed to Section 2.0

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
VSVG pseudo-typed lenti-virus	⊗ Yes O No	⊗ Yes O No	O Yes ⊗ No	0.04 L	academic	O 1 O 2 O 3 (2+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

Not using anymore yet

*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	O Yes ⊗ No		Not applicable
Rodent	⊗ Yes O No	Mouse brain	
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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	HEK 293(T)	ATCC (2/3)
Rodent	O Yes ⊗ No		
Non-human primate	O Yes ⊗ No		
Other (specify)	⊗ Yes O No	DT-40 Avian	academic (2+3)

*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 O 1 ⊗ 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES Ø 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? Ø YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5-alpha, RosettaBlue	pcDNA, pGEX	Invitrogen, GE Healthcare	NMDA receptors, TRP channels	protein expressed

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
lentivirus	pLB,	Addgene	shRNA	protein knockdown

not using yet

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? Ø YES O NO

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

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

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

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? O YES ⊗ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals O 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 ⊗ NO
- Pound source cats O YES ⊗ NO
- Cattle, sheep or goats O YES ⊗ NO
- Non- Human Primates O YES, please specify species _____ ⊗ NO
- Wild caught animals O YES, please specify species & colony # _____ ⊗ NO
- Birds O YES ⊗ NO
- Others (wild or domestic) O YES, please specify _____ ⊗ NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) tetrodotoxin
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 3-10 ug/kg

9.0 Import Requirements

9.1 Will the agent be imported? ⊗ YES, please give country of origin tetrodotoxin imported from Israel O NO
If no please proceed to Section 10.0

9.2 Has an Import Permit been obtained from HC for human pathogens? O YES ⊗ NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? O YES ⊗ NO

9.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ ⊗ NO

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

10.0 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
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 J J Mac Donald

11.0 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. 1 (2) 3 ~~(2/3)~~

11.2 Has the facility been certified by OHS for this level of containment?

YES, permit # if on-campus B10-RR1-0055 (2 - level 2)
NO As soon as the lab is setup
NOT REQUIRED it will be inspected

12.0 Procedures to be Followed

12.1 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. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE J J Mac Donald Date: October 24 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: E. K. Elder
Date: 8 Sept 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: A. Hawley
Date: Sept 4/09

Approval Number: B10 RR1 0055 Expiry Date (3 years from Approval) Sept 7, 2012

Special Conditions of Approval:

- Before unlisted work is started, a biohazard application and level 2 inspection is required
- Biosafety Requirements for Recombinant DNA Biological Agents to be followed

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

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
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 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 biohazards being used.

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

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

PRINCIPAL INVESTIGATOR	Robert Gros
SIGNATURE	
DEPARTMENT	Vascular Biology
ADDRESS	Robarts Research 4th floor
PHONE NUMBER	ext 24429 or 24428
EMERGENCY PHONE NUMBER(S)	519-719-0284
EMAIL	rgros@robarts.ca

Location of experimental work to be carried out: Building(s) Robarts Res. Ins Room(s) 4278/4274

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR and HSFO
GRANT TITLE(S): Regulators of G-protein-coupled receptor-mediated vascular responses.
Rapid vascular effects of steroids

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Bonan Liu
Qingming Ding
Jozef Chorazyczewski

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? Ø YES O NO
(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? O YES Ø 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:

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	PHAC or CFIA Containment Level
E. coli DH5a	O Yes Ø No	O Yes Ø No	O Yes Ø No	0.1L	Invitrogen	Ø1 O2 O3
Adenovirus	O Yes Ø No	O Yes Ø No	O Yes Ø No	0.5L	Microbix	O1 Ø2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*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? Ø YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	Ø Yes O No	Blood	Not applicable
Rodent	Ø Yes O No	Vascular	2009-037
Non-human primate	O Yes Ø No		
Other (specify)	O Yes Ø 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)*	Supplier / Source
Human	☒ Yes ○ No	HEK 293	ATCC
Rodent	☒ Yes ○ No	Rat/mouse vasc.SMCs	isolated from rodents
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 ○ 3

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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Volunteers/ patients	○ Yes ○ No ☒ Unknown		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No ○ Unknown		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ○ No ○ Unknown		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☒ 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 Transfected	Describe the change that results
E.coli DH5a	PDC315 PDC316	Invitrogen	GPR30, MR, GRK2, ACs	See appendix

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Adenovirus	Adeno-GFP, -MR -GPR30, -GRK2, -ACs	Microbix	MR, GPR30, ACs GRK2	See appendix

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ⊗ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ⊗ NO
- SV 40 Large T antigen O YES ⊗ NO
- E1A oncogene ⊗ YES O 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? ⊗ YES O 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 involving a biological agent? O YES ⊗ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

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

_____

_____

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

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø 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_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 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 Ø NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

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

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

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

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

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

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?

O YES, permit # if on-campus BIO-RRI-0031
O NO, please certify
O NOT REQUIRED for Level 1 containment

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 _____ Date: June 30/2010

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

_____

_____

_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

Express wound to bleed, wash with soap and water, then proceed to staff health

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: Susan Koval, Interim Chair
Date: July 9, 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: July 02, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: JStanley
Date: July 9, 2010

Approval Number: BIO-RRI-0031 Expiry Date (3 years from Approval): July 8, 2013

Special Conditions of Approval:

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

Description of Research Projects.

In our laboratory we utilize a variety of isolated tissues/cells, including primary and established cultures of rodent (rat and mouse) vascular smooth muscle cells as well as whole blood isolated from research volunteers and/or patients to examine the following:

a) The effect(s) of high blood pressure on cellular function is isolated cells from rodents and/or humans.

b) Regulation of isolated cells (rat, mouse or human) in response to steroid hormones

For many of these experiments we require adenoviral constructs in order to regulate the expression of various proteins/genes of interest in these isolated cells.

All of the adenoviral constructs listed in the Biohazardous Agents Registry Form have been generated using the Microbix adenovirus vector creation kit as previously described in some of our publications (Ding et al., 2009, Gros, et al., 2006; Gros, et al., 2007).

Briefly, rat or mouse vascular smooth muscle cells will be isolated via enzymatic digestion as previously described in detail (Gros, et al, 2006; Ding, et al., 2009; Gros, et al., 2007) and maintained in culture for various experiments and assays. Of note, live animals are only to be used a source of tissues and cells and will not be used for infection with the various adeno-viral constructs. For isolated human mononuclear leukocytes we utilize Ficoll-Hypaque separation protocol as previously described in detail (Gros, et al., 2007).

Cells maintained in culture are infected using the various adenoviral constructs. Briefly, cells are incubated with adenoviral constructs for 12-16h following which cells are washed and culture media replaced. Forty eight hours post-infection cells are utilized for various assays including arborization, contraction as well as western blotting.

REFERENCES:

Gros, R., Q. Ding, et al. (2006). "Adenylyl cyclase isoform-selective regulation of vascular smooth muscle proliferation and cytoskeletal reorganization." Circ Res **99**(8): 845-52.

Gros, R., S. Van Uum, et al. (2007). "Increased enzyme activity and beta-adrenergic mediated vasodilation in subjects expressing a single-nucleotide variant of human adenylyl cyclase 6." Arterioscler Thromb Vasc Biol **27**(12): 2657-63.

Ding, Q., R. Gros, et al. (2009). "Estradiol-mediated ERK phosphorylation and apoptosis in vascular smooth muscle cells requires GPR 30." Am J Physiol Cell Physiol **297**(5): C1178-87.

APPENDIX

Section 4.2

Transfection of gene:	Change that results
MR	Expression of mineralocortocoid receptor
GPR30	Expression of GPR30 receptor
ACs	Expression of Adenylyl Cyclase Isoforms
GRK2	Expression of G-protein-receptor kinase 2

Section 4.3

Adeno-mediatedgene transfer of:	Change that results
Adeno-GFP	Expression of green fluorescent protein
Adeno-MR	Expression of mineralocortocoid receptor
Adeno-GPR30	Expression of GPR30 receptor
Adeno-ACs	Expression of Adenylyl Cyclase Isoforms
Adeno-GRK2	Expression of G-protein-receptor kinase 2

Cell Biology

ATCC® Number:	CRL-1573™ [Order this Item]	Price:	$256.00

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 TH01: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	

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Material Transfer Agreement
Technical Support
Related Cell Culture Products

Login Required ▸
Product Information Sheet

This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments:

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D

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Canada

Adenovirus types 1, 2, 3, 4, 5 and 7 - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Adenovirus types 1, 2, 3, 4, 5 and 7***

SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

CHARACTERISTICS: *Adenoviridae*; non-enveloped, icosahedral virions, 70-90 nm diameter, doubled-stranded, linear DNA genome.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is associated with adenovirus infection

EPIDEMIOLOGY: Worldwide; seasonal in temperate regions, with highest incidences in the fall, winter and early spring; in tropical areas, infections are common in the wet and colder weather; annual incidence is particularly high in children; adenovirus types 4 and 7 are common among military recruits (ARD)

HOST RANGE: Humans

INFECTIOUS DOSE: >150 plaque forming units when given intranasally

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal -oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available; cidofovir has shown promise in the treatment of adenoviral ocular infections.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

PHYSICAL INACTIVATION: Sensitive to heat>56°C; unusually stable to chemical or physical agents and adverse pH conditions

SURVIVAL OUTSIDE HOST: Resistance to chemical and physical agents allows for prolonged survival outside of the body. Adenovirus type 3 survived up to 10 days on paper under ambient conditions; adenovirus type 2 survived from 3-8 weeks on environmental surfaces at room temperature

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

PROPHYLAXIS: None available

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Ten cases documented up to 1988

SOURCES/SPECIMENS: Respiratory secretions

PRIMARY HAZARDS: Ingestion; droplet exposure of the mucous membrane

SPECIAL HAZARDS: Contact with feces from infected animals

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

PROTECTIVE CLOTHING: Laboratory coat; gloves when skin contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

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

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

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

15. REGULATORY INFORMATION (CONT.)

Not classified

Component	EINECS Number
DIMETHYL SULFOXIDE	200-664-3

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
DIMETHYL SULFOXIDE	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES: None assigned.

PRODUCT SAFETY PHRASES: Not applicable.

PRODUCT CLASSIFICATION:

10. STABILITY AND REACTIVITY (CONT.)

degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Sulfur containing gases.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG

Target Organs: Blood. Eyes. Skin.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades slowly.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Gloves should be used as minimum hand protection.

Respiratory:
Use supplied-air respiratory equipment as required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalies. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO

6. ACCIDENTAL RELEASE MEASURES (CONT.)

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill.
Ventilate the contaminated area.
Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
Keep closed or covered when not in use.

Storage Procedures:
Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
DIMETHYL SULFOXIDE	Not established.	Not established.

Engineering Controls:
Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
Safety glasses should be the minimum eye protection.
Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash

4. FIRST AID MEASURES (CONT.)

glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Carbon dioxide Carbon monoxide Sulfur containing gases

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if absorbed.
****

Potential Health Effects:
Eye:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin:
Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage.
Upon prolonged or repeated exposure, harmful if absorbed through the skin. May cause minor systemic damage.

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.
No toxicity expected from inhalation.

Ingestion:
Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin:
Wash with soap and water. Get medical attention if irritation develops or persists.

Inhalation:
Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion:
Do not induce vomiting and seek medical attention immediately. Drink two