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

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

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

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

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

PRINCIPAL INVESTIGATOR	Zia A. Khan
DEPARTMENT	Pathology
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EMERGENCY PHONE NUMBER(S)	519-630-5151
EMAIL	Zkhan5@uwo.ca

Location of experimental work to be carried out: Building(s) **Dental Sciences Bldg** Room(s) **4011, 4004, 4020**

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

FUNDING AGENCY/AGENCIES: **CIHR, Canadian Diabetes Association (CDA)**

GRANT TITLE(S): **Molecular and Cellular Basis of Infantile Hemangioma Pathogenesis (CIHR), Vascular Stem Cells in Diabetic Complications (CDA)**

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

Name	UWO E-mail Address	Date of Biosafety Training
Emily Keats	ekeats@uwo.ca	Sept-2009
Samah Rafehi	srafehi@uwo.ca	Sept-2010
Rana Chakrabarti	rchakra@uwo.ca	May-2008

RESEARCH SUMMARY AND DESCRIPTION OF EXPERIMENTS CONDUCTED IN Dr. KHAN'S LABORATORY.

Location: Rooms 4004, 4011, and 4020 Dental Sciences Building.

Brief Description: Our research group will investigate the role of adult circulating and tissue stem cells in vascular repair and homeostasis. The cells will be isolated from various sources including human blood (LHSC/SJHC), human bone marrow (commercial), human tumour specimens (LHSC), and mouse blood/tissue specimens (UWO-DSB Animal Facility) by using antibody-coated magnetic beads (commercially available). We will culture the cells in growth media supplemented with fetal bovine serum and growth factors. These primary cells will then be subjected to cellular and molecular assays to investigate the behaviour of these adult stem cells *in vitro*. We intend to use bovine endothelial cells for co-culture experiments in which we will plate human and bovine cells together. The rationale is to use different species (that can be easily distinguished by specific-specific antibodies) to understand the effect of cell-cell contact on the differentiation process.

All techniques in the lab heavily rely on cell culture and cellular activity assays including proliferation, differentiation, growth, and migration. Molecular assays comprise of gene expression analyses, gene over-expression and knockdown, and protein analyses. Cells are also injected in athymic nude mice using matrix substrate (Matrigel; BD Biosciences) to study the behaviour in an *in vivo* setting.

1. Gene Knockdown/Transfections:

For gene knockdown, we will use small hairpin RNA (shRNA) in a lentiviral plasmid. These plasmids will be used only for stable transfection of our primary cells. We will not use the plasmids for stock preparation. Similarly, gene-overexpression will be achieved by full length cDNA of target gene in pCMV plasmid. The target genes for our studies are insulin-like growth factors (not oncogenic). All waste will be disinfected and then autoclaved. We will also use appropriate PPE. And finally, all work will be conducted in a biological safety cabinet.

2. Biological Specimens and Cell Isolation:

The procedure involving human and rodent specimens consists of cell isolation and culture. The specimens and the corresponding research approval status are given below.

Specimen	Source	REB/AUC Phase
Human blood	Healthy Volunteers	Approved
	Diabetic Patients[1]	Approved
Human blood/bone marrow mononuclear cells	Commercial	N/A
Human tissue	Hemangioma patients[2]	Approved
Mouse blood	Nu/nu mice	Approved
Explanted mouse tissue	Nu/nu & B6 mice	Approved
Bovine endothelial cells	Commercial	N/A

[1] *Blood samples from healthy volunteers will be collected at LHSC/SJHC.*

[2] *Blood samples from diabetic patients will be obtained through collaboration with Dr. Jeffrey L. Mahon (LHSC/SJHC)*

[3] *Hemangioma specimens will be obtained through collaboration with Drs. Nancy Chan (Pathology/LHSC) and Damir Matic (Plastic Surgery; LHSC).*

3. Animal Experiments:
We will investigate the function of primary cells (isolated from human blood or tumour specimens) in athymic nu/nu mice. Briefly, cells will be resuspended in Matrigel (BD Biosciences) and injected subcutaneously on the upper back of 6 week old mice. The explants will be harvested (at regular intervals starting at 7 days) and subjected to various assays including cell isolation and histochemical studies. Blood samples will also be taken from the mice to study the circulating cells. Finally, B6 mice will be used to isolate bone marrow for cell culture studies.

Please include a one page research summary or teaching protocol.

Please see research summary in the section above.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O 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? O YES O 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)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☑ YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☑ Yes O No	Blood (adult blood and cord blood), tumours, bone marrow, and other tissues (including skin, umbilical vein, umbilical artery, brain)	2008-019-04
Rodent	☑ Yes O No	Brain (endothelial cells)	N/A
Non-human primate	O Yes ☑ No		
Other (specify)	☑ Yes O No	Bovine aortic endothelial cells	N/A

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

See E-mail

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Commercial (US Biological, Stem Cell Tech, Allcells, Lonza, 3HBiomedical, and Promo Cell GmbH)	O Yes ☑ Unknown		O 1 ☑ 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes ☑ Unknown		O 1 ☑ 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes ☑ Unknown		O 1 ☑ 2 O 2+ O 3
Human Organs or Tissues (preserved)	Fixed tissue slides from Abcam, Ray Biotech, Imgenex Corporation	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 O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
N/A	*Lentiviral plasmid & pCMV plasmid (these plasmids are ready to be used for transfections and will not be used for produce virus)*	*Santa Cruz Biotech and Origene*	*A number of genes will be targeted*	*Transfection is expected to change the differentiation capacity of adult stem cells. These have been approved as of July 2009 (see attached)*

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
N/A				

* 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 O YES ☑ NO
- Known oncogenes O YES, please specify _____ ☑ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☑ NO

4.5 Will virus be replication defective? **N/A** O YES O NO

4.6 Will virus be infectious to humans or animals? **N/A** O 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? ☑ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used **Athymic nu/nu mice and B6 mice**

6.3 AUS protocol # **2008-019-04**

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

6.5 Will the agent(s) be shed by the animal: O YES ☑ NO, please justify:
We will inject cells that have been transfected with plasmids (no virus) and the cells will be localized under the skin. This modification is not oncogenic and the cells will not be shed from the animals.

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Will live animals be used? O YES O No

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

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

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

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ☑ 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 details:_____ O NO

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

9.0 Insects

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____

_____

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

_____

_____

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

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

_____

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

_____

_____

_____

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

_____

_____

11.0 Import Requirements

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

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

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

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

☑ YES, date of most recent biosafety inspection: 07/10/2010
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0191**

14.0 Procedures to be Followed

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

Most of the procedures being carried out in our laboratory are routine tasks and pose minimal risk to the personnel. All lab personnel undergo training (through UWO workshops and by the PI) prior to initiating any projects and experiments. These training modules include locating safety stations (eye wash, safety showers, fire extinguishers, and spill kits), attending workshops (employee health and safety orientation, biosafety, and laboratory and environmental/waste management safety), keeping WHMIS training up-to-date, familiarizing with general lab safety guidelines (url link are provided to the personnel), and using personal protection equipment. All personnel are also familiarized with proper response to minor and major spills and other accidents.

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

In an event of such injury/exposure, the personnel are 1) wash the site with copious amount of water, 2) to report the incident immediately to the PI, 3) seek medical attention through the workplace health, and 4) provide as much information as possible. All incident records and action taken will be kept in the laboratory.

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

SIGNATURE _____ Date: 01/04/2011

15.0 Approvals

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

2) Safety Officer for the University of Western Ontario

SIGNATURE: _____
Date: _____

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

SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

Subject: Questions
From: Zia Khan <Zia.Khan@schulich.uwo.ca>
Date: Thu, 13 Jan 2011 14:29:59 -0500
To: "<Jennifer Stanley" <jstanle2@uwo.ca>

E-mail

Jennifer -

here are the answers to your questions:
Q1. Bovine Aortic Endothelial Cells (Source: Lonza, Catalogue BW-6002). The information sheet is the same as the one submitted in the previous registry mod. These cells are obtained commercially and will only be used in culture experiments (no in vivo experiments).
Q2. Rat Brain Endothelial Cells (Source Cell Applications, Catalogue R840-05). Please see attached. These cells are obtained commercially and will not be used in animal experiments.
Q3. For the human cells, I included the AUS protocol number because these human cells will be injected in mice. These cells are obtained from various sources (please see research summary for more details).

If you have any further questions - please do let me know.
Many thanks
ZK

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Rat Brain ECs.pdf	**Content-Type:**	application/pdf
	Content-Encoding:	base64

Rat Brain Microvascular Endothelial Cells: RBMVEC

(Enlarge Image)

Rat Brain Microvascular Endothelial Cells, RBMVEC

Rat Brain Microvascular Endothelial Cells (RBMVEC) are derived from the brain of adult Sprague Dawley rat. The cells are cryopreserved at second passage. RBMVEC can be cultured and propagated 16 population doublings. HIV-1 Tat protein alters the tight junction protein expression and distribution in cultured brain endothelial cells. It is believed such alteration lead to disturbances of the blood-brain barrier (BBB) integrity and contributes to HIV trafficking in to the brain[1]. Endothelial cell-derived monocyte-chemoattractant protein-1 (MCP-1) plays a key role in leukocyte recruitment across the blood-brain and blood-retinal barriers *in vivo*[2]. Three-dimensional model indicates that dipyridamole can improve BBB function[3]. Mainteance of BBB function during and following normoxic-cormoglycemic flow cessation appears to depend on intact nitric oxide signaling and IL-6 release[4]. Expression of metabotropic glutamate receptors may modulate synaptic transmission and determine microvascular function and dysfunction[5]. VEGF mRNA and VEGF receptor F1K-1/KDR are both higher in neonatal than in adult microvessels suggesting that microvascular cell turnover continues in the adult brain[6]. Hypoxia increases the aparacelluair flux across the cell monolayer via the release of VEGF, which in turn leads to the dislocalization, decreased expression and enhanced phosphorylation of Zonula occludens-1 (ZO-1)[7].

1. Andras IE, et al, J Neurosci Res. 2003 Oct 15; 74(2): 255.
2. Harkness, KA, et al, Neuroimmunol. 2003 Sep; 142(1-2):1.
3. Parkinson FE, et al, Brain Res. 2003 Aug. 8; 980(2) 233.
4. Krizanac-Bengez, L, et al, Brain Res. 2003 Jul. 11: 977(2): 239.
5. Gillard, SE, et al, J Comp. Neurol. 2003 Jun. 30; 461(3): 317.
6. Hoehn, BD, et al, Brain Res. Mol Brain Res. 2002 May 30; 101(1-2): 103.
7. Fischer S, et al, Microvasc Res. 2002 Jan; 63(1): 70.

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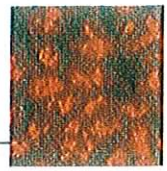

Clonetics® Bovine Endothelial Cells

Introduction

Lonza now compliments its human primary derived endothelial cells with several bovine endothelial cultures. The tissue origin of the bovine cells are aorta, pulmonary artery and coronary artery.

Aortic endothelial cells can be purchased as single donors, one aorta per lot, or as pooled donors, three to five aortas per lot. Pulmonary artery and coronary artery cells are available only as single donor lots. Bovine aortic and pulmonary artery endothelial cells are isolated and frozen in first passage. The bovine coronary artery endothelial cells are frozen in third passage. Following cryopreservation, cells are quality tested for: viability, seeding efficiency, growth rate, morphology and purity.

Helpful Hints

- A cryopreserved amp should be seeded into multiple T-25 flasks. Optimal performance is observed when cells are initially seeded into smaller flasks.
- Thaw and plate cells quickly. Do NOT centrifuge!
- Incubate cells overnight and change medium within 24 hours to remove residual DMSO.
- Continue to change medium every other day.

Cell System Components

- One Bovine Endothelial Cell Product (Cryopreserved or Proliferating)
- One Endothelial Cell Medium BulletKit® - 500 ml Clonetics® EGM®-MV BulletKit® (CC-3125) contains one 500 ml bottle of Endothelial Cell Basal Medium and the following growth supplements: BBE, 2 ml; hEGF, 0.5 ml; Hydrocortisone, 0.5 ml; FBS, 25 ml; GA-1000, 0.5 ml
- One ReagentPack™ (CC-5034) Containing:

Trypsin/EDTA	100 ml
Trypsin Neutralizing Solution	100 ml
HEPES Buffered Saline Solution	100 ml

Characterization of Cells

Routine characterization of bovine endothelial cells includes positive staining for acetylated LDL uptake and morphological observation from cryopreservation through confluence.

Performance

Recommended seeding density for subculture	2,500 - 5,000 cells/cm^2
Typical time from subculture to confluent monolayer	5 - 9 days

Quality Control

All cells are performance assayed and test negative for bacteria, yeast and fungi. Cell viability and morphology is measured after recovery from cryopreservation. Clonetics® Media are formulated for optimal growth of specific types of normal human cells. Each lot of medium is tested for the support of cell viability and proliferative capacity. Certificates of Analysis (CA) for each cell strain are shipped with each order. CA for all other products are available upon request.

Ordering Information

BW-6001	bAEC, Bovine Aortic Endothelial Cells, cryopreserved	≥500,000 cells
AC-6001T25	bAEC, Bovine Aortic Endothelial Cells, proliferating	T-25 Flask
AC-6001T75	bAEC, Bovine Aortic Endothelial Cells, proliferating	T-75 Flask
AC-6001W96	bAEC, Bovine Aortic Endothelial Cells, proliferating	96-well Plate
BW-6002	bAEC, Bovine Aortic Endothelial Cells,	≥500,000 cells

	pooled, cryopreserved	
AC-6002T25	bAEC, Bovine Aortic Endothelial Cells, pooled, proliferating	T-25 Flask
AC-6002T75	bAEC, Bovine Aortic Endothelial Cells, pooled, proliferating	T-75 Flask
AC-6002W96	bAEC, Bovine Aortic Endothelial Cells, pooled, proliferating	96-well Plate
BW-6004	bPAEC, Bovine Pulmonary Artery Endothelial Cells, cryopreserved	≥500,000 cells
AC-6004T25	bPAEC, Bovine Pulmonary Artery Endothelial Cells, proliferating	T-25 Flask
AC-6004T75	bPAEC, Bovine Pulmonary Artery Endothelial Cells, proliferating	T-75 Flask
AC-6004W96	bPAEC, Bovine Pulmonary Artery Endothelial Cells, proliferating	96-well Plate
BW-6005	bCAEC, Bovine Coronary Artery Endothelial Cells, cryopreserved	≥500,000 cells
AC-6005T25	bCAEC, Bovine Coronary Artery Endothelial Cells, proliferating	T-25 Flask
AC-6005T75	bCAEC, Bovine Coronary Artery Endothelial Cells, proliferating	T-75 Flask
AC-6005W96	bCAEC, Bovine Coronary Artery Endothelial Cells, proliferating	96-well Plate
CC-3125	EGM®-MV BulletKit®, EBM® plus SingleQuots® of Growth Supplements	500 ml
CC-3121	EBM®, Endothelial Basal Medium	500 ml
CC-3129	EBM®-Phenol Red Free, EBM® w/o Phenol Red	500 ml
CC-4143	EGM®-MV SingleQuots®, Formulates EBM® to EGM®-MV	
CC-5034	ReagentPack™	
	Trypsin Neutralizing Solution	100 ml
	Trypsin/EDTA Solution	100 ml
	HEPES Buffered Saline Solution	100 ml

Lonza

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells only if Clonetics® Media are used, and the recommended protocols are followed. Cryopreserved bovine endothelial cells are assured to be viable and functional when thawed and maintained properly.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.

Lonza

Printed on, 19-Jan-2009 16:58

CERTIFICATE OF ANALYSIS

Product Code: 8W-6002
Product: bAEC Bov Aortic Endo EGM-MV,pooled, cryo amp

Lot Number: 0000088927
Manufacture Date: 25-Aug-2008

TEST (Method)	SPECIFICATIONS Min.	Max.	Results
Tissue Acquisition Number	***	***	P805
Donor Screen Information:			
Age	***	***	N/A
Race	***	***	N/A
Sex	***	***	UNKNOWN
Cell Type	***	***	BAEC
Cell Strain Calculations:			
Date of Cryopreservation	***	***	25 AUG 2008
Cell Passage			1
Cell Count (Cells/ml)	>= 500,000	***	542000
Viability Tryp.Blue Exclusion	>= 70%	***	83 %
Total Population Doublings	For Info Only	***	7
Seeding Efficiency	>= 20%	***	50 %
Doubling Time (hours)	15	48	15
QC Evaluation Medium			EGM MV
Sterility - Amp	***	***	Negative
Direct Plating (Mycoplasma)	***	***	Negative
Acetylated LDL Uptake Staining	***	***	Pass

This lot has been isolated from human tissue obtained under "informed consent". This lot has been tested in accordance Lonza's test procedures and sampling plans. Reported test results are within the limits of Lonza's current test procedures. This is to certify that all bovine material used in the production of this lot was collected in the contiguous 48 United States. The product was obtained only from USDA inspected facilities where animals receive ante and postmortem inspection and were found free of contagious disease. Details concerning the use of our cell and media products can be downloaded from our website at www.lonza.com.

This lot has been reviewed by Quality Assurance for compliance with requirements of Lonza's Quality System. This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.

-------- Original Message --------
Subject: Re: Use of bovine aortic endothelial cells - containment level request
Date: Mon, 28 Sep 2009 08:23:29 -0400
From: ImportZoopath <ImportZoopath@inspection.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

In this specific case, since these probably are uninfected products, I would suggest you contact your CFIA area office if you wish to import these products.

As for containment, it appears these are not derived from materials (speficied risk material - srm) with suspected risk of infectivity - therefore, the risk of it to contain BSE is very low to none. I would not consider them to be a hazard else than usual precautions applicable to working with any type of cell lines.

Again, Area office knows more than I about which bovine tissues are at a risk for SRM. You may want to contact them to get the final answer on it.

Have a nice day,

Cinthia Labrie

Jennifer Stanley <jstanle2@uwo.ca> 2009-09-18 17:10 >>>

Hi there
Can you give me some advice on the containment level required for the use of these bovine endothelial cells? I have attached the supplier information.
Thanks!
Jennifer

Office of Biohazard Containment & Safety, CFIA | Bureau du confinement des biorisques et de la sÃ©curitÃ©, ACIA
Government of Canada | Gouvernement du Canada
59 Camelot, Ottawa ON K1A0Y9
Phone/TÃ©l.: (613) 221-7068
Fax/ TÃ©lÃ©c.: (613) 228-6129
ImportZoopath@inspection.gc.ca

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

July 16, 2009

Dear Biosafety Committee Members,

We have recently added lentiviral plasmids encoding small RNA fragments (shRNA for gene knockdown) and mammalian pCMV6 expression plasmids encoding full length human cDNA (for gene expression) to our permit (UWO-BIO-0191). We would like to request you to allow our lab to be designated as BSL2. We understand that the risks with lentiviral vectors include 1) the potential to generate replication-competent virus particles, and 2) oncogenesis. However, we would like to request you to consider the nature of the vector system and the application/use when designating the containment level for our lab. In our lab, we will use the vector system in which 1) the vector and packaging functions are separated (3rd generation biosafety), and 2) the human gene has not been reported to be oncogenic (in the case of pCMV6). We will **not** use the plasmids for viral particle generation or transfection of producer/packaging cells. In fact, we have submitted the catalogue numbers for the plasmid we will be using (please see the modification form and attached appendices; catalogue numbers are also listed below). These items do not contain the packaging plasmids necessary for producing virus particles. We are also forwarding the email from Santa Cruz Biotechnology that states the nature of the vector system (3rd generation; multiple plasmids required to produce viral particles). Finally, our experiments are very similar to transient transfection of primary cells with siRNA which requires BSL2.

If you have any questions, please do contact me.

Zia A. Khan, PhD
Department of Pathology, UWO
Tel 519-661-2111 x81562

Catalogue Number	Vendor	Description	Use
SC320234	Origene	IGF2 cDNA in pCMV6	Transfection only
sc-29358-sh	Santa Cruz	IGF1R shRNA plasmid	Transfection only
sc-37193-sh	Santa Cruz	IGF1 shRNA plasmid	Transfection only
sc-39576-sh	Santa Cruz	IGF2 shRNA plasmid	Transfection only
sc-37118-sh	Santa Cruz	IGFR2 shRNA plasmid	Transfection only
sc-108083	Santa Cruz	copGFP control plasmid	Transfection only

Subject: Re: Biosafety approval: Khan
From: Zia Khan <Zia.Khan@schulich.uwo.ca>
Date: Tue, 26 May 2009 15:09:09 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Thanks Jennifer:

I would like to express one cDNA using lentiviral plasmid. The gene is insulin-like growth factor-2 (Origene; Catalogue SC320234).

For shRNA expression experiments, I will be using shRNA targetting 4 different genes. These include
1) insulin-like growth factor-1 (Santa Cruz; Catalogue sc-37193-sh)
2) insulin-like growth factor-2 (Santa Cruz; Catalogue sc-39576-sh)
3) insulin-like growth factor receptor-1 (Santa Cruz; sc-29358-sh)
4) insulin-like growth factor receptor-2 (Santa Cruz; sc-37118-sh)

These experiments also require a control plasmid. I plan to use copGFP Control plasmid (Santa Cruz; sc-108083).

Besides sc-37118-sh, I have found datasheets for all of the plasmids (please see attached).
If you need more information, please do let me know.
Many Thanks
ZK

Zia A. Khan, PhD
Assistant Professor
Department of Pathology
Schulich School of Medicine & Dentistry
University of Western Ontario

4011 - Dental Sciences Building
1151 Richmond Street
London, Ontario N6A 5C1

Tel (519) 661-2111 Ext 81562
Fax (519) 661-3370

sc-37193-sh.pdf **Content-Type:** application/pdf
Content-Encoding: base64

sc-39576-sh.pdf **Content-Type:** application/pdf
Content-Encoding: base64

sc-108083.pdf **Content-Type:** application/pdf
Content-Encoding: base64

SC320234.pdf	Content-Type:	application/pdf
	Content-Encoding:	base64

sc-29358-sh.pdf	Content-Type:	application/pdf
	Content-Encoding:	base64

SANTA CRUZ BIOTECHNOLOGY, INC.

IGF-I shRNA Plasmid (h): sc-37193-SH

BACKGROUND

Insulin-like growth factor-I, or IGF-I, is an ubiquitous peptide that acts in both an autocrine and paracrine fashion to stimulate the growth of vascular smooth muscle cells. In addition, IGF-I regulates renal function, growth and repair, is critically involved in bone formation and resorption, and has been implicated in mediating aspects of the immune response. IGF function is modulated by at least six circulating IGF-binding proteins, designated IGFBP1-6, which associate with the soluble growth factor. While the function of IGF-II is less well understood, overexpression of the protein in mice suggests that IGF-II may play a regulatory role in insulin sensitivity and glucose uptake. Both IGF-I and IGF-II exert their biological effects through a common receptor, designated IGF-IR. Like the insulin receptor, IGF-IR is composed of two extracellular α chains and two signal transducing β chains cross-linked by disulfide bonds.

REFERENCES

1. Rabkin, R., et al. 1995. Expression of the genes encoding the rat renal insulin-like growth factor-I system. J. Am. Soc. Nephrol. 6: 1511-1518.
2. Hayden, J.M., et al. 1995. The insulin-like growth factor system and the coupling of formation to resorption. Bone 17: 93S-98S.
3. Auernhammer, C.J. and Strasburger, C.J. 1995. Effects of growth hormone and insulin-like growth factor I on the immune system. Eur. J. Endocrinol. 133: 635-645.
4. Motani, A., et al. 1995. Insulin-like growth factor binding protein-1 inhibits arterial smooth muscle cell proliferation *in vitro* but does not reduce the neointimal response to balloon catheter injury. Atherosclerosis 118: 57-66.
5. Delafontaine, P., et al. 1996. G protein-coupled and tyrosine kinase receptors: evidence that activation of the insulin-like growth factor-I receptor is required for Thrombin-induced mitogenesis of rat aortic smooth muscle cells. J. Clin. Invest. 97: 139-145.

CHROMOSOMAL LOCATION

Genetic locus: IGF1 (human) mapping to 12q23.2.

PRODUCT

IGF-I shRNA Plasmid (h) is a pool of 2 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see IGF-I siRNA (h): sc-37193 and IGF-I shRNA (h) Lentiviral Particles: sc-37193-V as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and its reaction products and derivatives for research purposes conducted in the buyer's laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product for commercial purposes. Commercial purposes

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

IGF-I shRNA Plasmid (h) is recommended for the inhibition of IGF-I expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

IGF-I (H-70): sc-9013 is recommended as a control antibody for monitoring of IGF-I gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or Immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000). Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-FR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IGF-I gene expression knockdown using RT-PCR Primer IGF-I (h)-PR: sc-37193-PR (20 µl, 527 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

SANTA CRUZ BIOTECHNOLOGY, INC.

IGF-II shRNA Plasmid (h): sc-39576-SH

BACKGROUND

The insulin gene family, comprised of insulin, relaxin and insulin-like growth factors I and II (IGF-I and IGF-II), represents a group of structurally related polypeptides whose biological functions have diverged. The IGFs, or somatomedins, constitute a class of polypeptides that have a key role in pre-adolescent mammalian growth. IGF-I and -II are critical regulators of cell proliferation and differentiation. Most of the growth promoting properties of both ligands are mediated by the IGF-I receptor (IGF-IR). IGF-I and -II, respectively known as somatomedin C and somatomedin A, are single chain polypeptides which share an amino acid sequence homology of about 47% with insulin. IGF-I expression is regulated by growth hormone and mediates postnatal growth, while IGF-II is induced by placental lactogen during prenatal development. IGF-II is a fetal growth factor, influenced by placental lactogen and abundantly expressed by placental trophoblasts. IGF-II and IGF-binding protein 1 (IGFBP1) gene variants are associated with overfeeding-induced metabolic changes. The human IGF-II gene maps to chromosome 11p15.5, encoding a 180 amino acid protein which is the precursor to IGF-II.

REFERENCES

1. Bell, G.I., et al. 1984. Sequence of a cDNA clone encoding human pre proinsulin like growth factor II. Nature 310: 775-777.
2. Dull, T.J., et al. 1984. Insulin-like growth factor II precursor gene organization in relation to insulin gene family. Nature 310: 777-781.
3. Rotwein, A.M., et al. 1993. Structural analysis of the human insulin-like growth factor-II P3 promoter. Biochem. J. 289: 133-139.
4. Ukkola, O., et al. 2001. Insulin-like growth factor 2 (IGF2) and IGF-binding protein 1 (IGFBP1) gene variants are associated with overfeeding-induced metabolic changes. Diabetologia 44: 2231-2236.

CHROMOSOMAL LOCATION

Genetic locus: IGF2 (human) mapping to 11p15.5.

PRODUCT

IGF-II shRNA Plasmid (h) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see IGF-II siRNA (h): sc-39576 and IGF-II shRNA (h) Lentiviral Particles: sc-39576-V as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only, whether the buyer is an academic or for-profit entity. The buyer cannot sell or otherwise transfer this product (h) its components or derivatives made using this product or its components to a third party or otherwise use this product or its components or derivatives made using this product for commercial purposes.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

IGF-II shRNA Plasmid (h) is recommended for the inhibition of IGF-II expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

GENE EXPRESSION MONITORING

IGF-II (N-20): sc-1415 is recommended as a control antibody for monitoring of IGF-II gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:50,000). Cruz Marker™ Molecular Weight Standards: sc-2035. TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IGF-II gene expression knockdown using RT-PCR Primer: IGF-II (h)-PR: sc-39576-PR (20 µl, 455 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com or our catalog for general protocols and support products.

copGFP Control Plasmid: sc-108083

BACKGROUND

Santa Cruz Biotechnology, Inc. currently offers more than 49,000 target specific shRNA plasmids that encode 19-25 nucleotide (plus hairpin) shRNAs designed to knock down a wide variety of proteins. For each shRNA plasmid DNA product, we offer an appropriate control antibody for confirmation of targeted mRNA silencing by Western Blotting or immunofluorescence. We also offer non-targeted Control shRNA Plasmids. In addition, we offer the copGFP Control Plasmid, which contains the full-length copGFP gene with optimized human codons for high level expression of the fluorescent protein from the CMV promoter in mammalian cells. The copGFP marker is a novel natural green monomeric GFP-like protein from copepod (*Pontellina* sp.). The copGFP protein is a non-toxic, non-aggregating protein with fast protein maturation. Highly stable at a wide range of pH (pH 4-12), the copGFP protein does not require any additional cofactors or substrates. The copGFP protein has very bright fluorescence that exceeds at least 1.3 times the brightness of EGFP, the widely used *Aequorea victoria* GFP mutant. The copGFP protein emits green fluorescence with the following characteristics:

Maximum emission wavelength: 502 nm
Maximum excitation wavelength: 482 nm
Quantum yield: 0.6
Extinction coefficient: 70,000 M-1 cm-1

Due to its exceptional properties, copGFP is an excellent fluorescent marker that can be used to monitor delivery of shRNA lentiviral constructs into cells.

PRODUCT

copGFP Control Plasmid is a lentiviral vector plasmid that encodes the copGFP fluorescent protein in mammalian cells. copGFP Control Plasmid is provided as transfection-ready purified plasmid DNA. Each vial contains 20 µg lyophilized shRNA plasmid DNA sufficient for up to 20 transfections when resuspended as directed below. Also see copGFP Control Lentiviral Particles: sc-108084 as an alternate control for use in transduction-based experiments.

STORAGE AND RESUSPENSION

Store lyophilized copGFP Control at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized copGFP Control in 200 µl of the deionized water provided. Resuspension of copGFP Control in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

copGFP Control Plasmid is recommended for use as a control to monitor and optimize transfection efficiency, thus assuring satisfactory levels of targeted shRNA-knockdown. After transfection, cells stably expressing copGFP may be isolated via puromycin selection.

GFP (B-2): sc-9996 is recommended as a control antibody for detection of copGFP.

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

SUPPORT REAGENTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
shRNA Plasmid Transfection Reagent	sc-108061	[illegible]	0.2 ml 50-100 transfections
shRNA Plasmid Transfection Medium	sc-108062	[illegible]	20 ml
Control shRNA Plasmid-A	sc-108060	[illegible]	20 µg 20 transfections
Control shRNA Plasmid-B	sc-108065	[illegible]	20 µg 20 transfections
Control shRNA Plasmid-C	sc-108066	[illegible]	20 µg 20 transfections

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

IGF2 (NM_001007139) Human cDNA Clone

Specifications		Related Products	Product Manual	FAQs	
Cat. No.	Ref. ID	Description		Price	Delivery
SC325234	NM_001007139	Homo sapiens insulin-like growth factor 2 (somatomedin A) (IGF2), transcript variant 2 as transfection-ready DNA NM_001007139.3		$580	Immediate

Please select amount: 10ug

Tagged ORF | shRNA | Primer Pair | Antibody Search

OriGene TrueClone Data

Vector: pCMV6-AC

Insert Size:

Sequence Data:	Edited Nucleotide Sequence 5' Read Nucleotide Sequence
OTI Annotation:	This TrueClone is provided through our Custom Cloning Process that includes sub-cloning into OriGene's pCMV6 vector and full sequencing to provide a non-variant match to the expected reference without frameshifts, and is delivered as lyophilized plasmid DNA
OTI Disclaimer:	Our molecular clone sequence data has been matched to the reference identifier above as a point of reference. Note that the complete sequence of our molecular clones may differ from the sequence published for this corresponding reference, e.g., by representing an alternative RNA splicing form or single nucleotide polymorphism (SNP)
Product Components:	This cDNA clone is shipped in a 2-D bar-coded Matrix tube as dried plasmid DNA. The package also includes 100 pmols of both the corresponding 5' and 3' vector primers in separate vials. Every lot of primer is tested to provide clean sequencing of OriGene TrueClones

Reference Data

RefSeq: NM_001007139.3, NP_001007140

RefSeq Size: 5139

RefSeq ORF: 542

Synonyms: C11orf43; FLJ22066; FLJ44734; INSIGF; pp9974

LocusID: 3481

Cytogenetic: 11p15.5

Summary: This gene encodes a member of the insulin family of polypeptide growth factors that is involved in development and growth. It is an imprinted gene and is expressed only from the paternally inherited allele. It is a candidate gene for eating disorders. There is a read-through, INS-IGF2, which aligns to this gene at the 3' region and to the upstream INS gene at the 5' region. Alternatively spliced transcript variants encoding either the same or different isoform, have been found for this gene. [provided by RefSeq]

Transcript Variant: This variant (2) contains two alternate 5' non-coding exons, therefore, has a different 5' UTR compared to variant 1. Transcript variants 1 and 2 encode the same isoform (1)

SANTA CRUZ BIOTECHNOLOGY, INC.

IGF-IRα/β shRNA Plasmid (h): sc-29358-SH

BACKGROUND

Receptor tyrosine kinases (RTKs) are transmembrane molecular scaffolds that influence cellular processes, including cell migration, metabolism, survival, proliferation and differentiation. Insulin-like growth factor-I receptor (IGF-IR) is an RTK that stimulates growth in many different cell types, blocks apoptosis, acts as an intermediate of many growth hormone responses and may stimulate the growth of some types of cancer. The IGF-IR cognate ligand, Insulin-like growth factor-I (IGF-I), promotes association of IGF-IR with Shc, GRB2 and Sos 1, which initiates Ras and ERK kinase cascades, thereby modifying transcription factor activity, such as activation of the Elk transcription factors. The modular phosphotyrosine binding (PTB) domains of Insulin receptor substrate (IRS)-1 and -2 can associate with active IGF-IR and initiate phosphatidylinositol 3-kinase-dependent downstream signals. The human IGF-IR gene maps to chromosome 15q26.3 and encodes a 1,376 amino acid precursor protein that cleaves into α and β subunits. The human IGF-IIR gene maps to chromosome 6q26 and encodes a 2,491 amino acid transmembrane protein.

REFERENCES

1. Frattali, A.L., et al. 1993. Molecular defects of Insulin/IGF-I receptor transmembrane signaling. Ann. N.Y. Acad. Sci. 687: 77-89.
2. Keller, S.R., et al. 1993. Insulin and IGF-I signaling through the Insulin receptor substrate 1. Mol. Reprod. Dev. 35: 346-352.
3. De Meyts, P., et al. 1995. Mechanism of Insulin and IGF-I receptor activation and signal transduction specificity. Receptor dimer cross-linking, bell-shaped curves, and sustained versus transient signaling. Ann. N.Y. Acad. Sci. 766: 388-401.
4. Song, R.X., et al. 2001. The role of Shc and Insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor alpha to the plasma membrane. Proc. Natl. Acad. Sci. USA 101: 2076-2081.
5. Mitsiades, C.S., et al. 2004. Inhibition of the Insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell 5: 221-230.
6. Salatino, M., et al. 2004. Inhibition of *in vivo* breast cancer growth by antisense oligodeoxynucleotides to type I Insulin-like growth factor receptor mRNA involves inactivation of ErbBs, PI-3K/Akt and p42/p44 MAPK signaling pathways but not modulation of progesterone receptor activity. Oncogene 23: 5161-5171.
7. Broussard, S.R., et al. 2004. IL-1β impairs Insulin-like growth factor I-induced differentiation and downstream activation signals of the Insulin-like growth factor I receptor in myoblasts. J. Immunol. 172: 7713-7720.
8. Harvell, K., et al. 2004. Insulin receptor substrate-1/SHP-2 interaction, a phenotype-dependent switching mechanism of Insulin-like growth factor-I signaling in vascular smooth muscle cells. J. Biol. Chem. 279: 16807-16816.

PROTOCOLS

See our Web site at www.scbt.com or our catalog for detailed protocols and support products.

CHROMOSOMAL LOCATION

Genetic locus: IGF1R (human) mapping to 15q26.3.

PRODUCT

IGF-IRα/β shRNA Plasmid (h) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see IGF-IRα/β siRNA (h) sc-29358 and IGF-IRα/β shRNA (h) Lentiviral Particles: sc-29358-V as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

APPLICATIONS

IGF-IRα/β shRNA Plasmid (h) is recommended for the inhibition of IGF-IRα expression in human cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA, each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IGF-IRα gene expression knockdown using RT-PCR Primer: IGF-IRα (h)-PR: sc-29358-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

Material Safety Data Sheet

Section 1. Product and Company Identification

Product Name: TrueClone cDNA clones
Catalog Number:
Manufacturer: OriGene Technologies, Inc. Six Taft Court, Suite 100, Rockville, MD 20850, USA
Contact: 888-267-4436 (Tel) or 301-340-8606 (Fax), Info@origene.com, www.origene.com
Validation Date: 09/29/04

MSDS# OTITC0904
Component/Item (and Parts number if listed)
Complementary DNA (cDNA) clones dried in individual eppendorf tubes

Section 2. Composition and Information on Hazardous (OSHA) Ingredients

All components of the products are considered non-hazardous. As yet, the chemical, physical, and toxicological properties of these products have not been thoroughly investigated. These products are provided as dried plasmid DNA and this MSDS is written to apply to general reagents.

Section 3. Hazards Identification

Review approved and the most current institutional guideline, protocol, SOP(s) and MSDS(s) for the proper handling of institutional materials/equipment associated with the use of this BCI product.
Primary Routes of Entry:
Skin Absorption (No); Dermal/skin contact (Yes); Eye contact (Yes); Inhalation (No); Ingestion (Yes); Chronic Exposure (No).
Medical Conditions Aggravated by Exposure: Not available.
Potential Acute Health Effects: Adverse health effects are not expected from the use of this product.
Carcinogenic Effects: Not listed by NTP, IARC or OSHA.
Mutagenic Effects: Not available. *Teratogenic Effects:* Not available.

Section 4. First Aid Measures

Emergency First Aid Procedures: Wash affected area with water for at least 15 minutes. See physician

Section 5. Fire Fighting Measures

Special Fire Fighting: N/A

Section 6. Accidental Release Measures

If released or spilled Absorb on neutral material. Wash area thoroughly.

Section 7. Handling and Storage

See User's Manual for storage information

Section 8. Exposure Controls and Personal Protection

Effects of Overexposure: N/A *Respiratory Protection:* None needed
Ventilation General ventilation *Protective Glove:* General lab safe gloves
Eye Protection: Use general eye protection-goggles.
Handling and Storage: Wear appropriate protective clothing and gloves. Store in cold

Section 9. Physical and Chemical Properties

Appearance: Solution.
Boiling Point: N/A
Specific Gravity: N/A
Vapor Density & Pressure: N/A
Solubility in H2O: Soluble

Section 10. Stability and Reactivity

Stability and Reactivity: The product is stable
Incompatibility: N/A
Hazardous Decomposition Products: N/A

Section 11. Toxicological Information

N/A

Section 12. Ecological Information

The product itself and its products of degradation are not toxic.

Section 13. Disposal Considerations

Please consult local, state and federal regulation on additional guidance on disposal.

Section 14. Transport Information

Contact OriGene for all transport information.

Section 15. Regulatory Information

N/A

Section 16. Other Information

Validated by OriGene Safety Office on 09/29/2004. Verified by OriGene Administration and Printed on 09/29/2004.

Notice to Reader
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N/A - Not applicable or no information available.

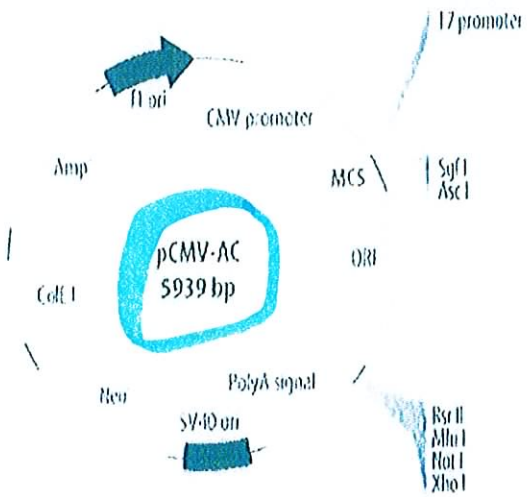
pCMV6-AC Non-Tagged PrecisionShuttle Vector

Specifications | Custom Cloning | Product Manual | FAQs

Catalog No.	Description	Price
PS100020	PrecisionShuttle pCMV6-A (non-tagged) Destination Vector	$320

Features:

- This vector is essentially the same as the pCMV6-Entry vector, except for the absence of the C-terminal Myc-DDK tag.
- ORF cloned into this vector at SgfI and PsrII sites will express the ORF-encoded protein plus two additional amino acids at the C-terminus, T and R.
- The vector itself does not contain additional tag. If the insert is shuttled from pCMV6-Entry via SgfI/AscI and PsrII/MluI, it will express non-tagged protein. If the insert is shuttled from the pCMV6-Entry vector using SgfI/AscI and PmeI, then it will express Myc-DDK tagged protein.



pCMV6-A

KozakATG

SgfI AscI BamHI XhoI PsrII MluI NotI XhoI PmeI FseI

Schematic of the multiple cloning sites:

Download full vector sequence

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 05/07/2009
Date Updated: 07/13/2005
Version 1.0

Section 1 - Product and Company Information

Product Name	MISSION PLKO.1-PURO CONTROL VECTOR
Product Number	SHC001
Brand	SIGMA
Company	Sigma-Aldrich Canada, Ltd
Address	2149 Winston Park Drive Oakville ON L6H 6J8 CA
Technical Phone:	9058299500
Fax:	9058299292
Emergency Phone:	800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
MISSIONTM PLKO.1-PURO CONTROL VECTOR	None	No

Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
TRIS-EDTA BUFFER 100X CONCENTRATE	None	1	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Irritant.
Irritating to eyes, respiratory system and skin.

HMIS RATING

HEALTH: 2
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING

HEALTH: 2
FLAMMABILITY: 0
REACTIVITY: 0

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE
In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE
In case of contact, immediately flush eyes with copious amounts of water for at least 15 minutes.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Absorb on sand or vermiculite and place in closed containers for disposal. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe vapor. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE
Store at -20°C

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Mechanical exhaust required. Safety shower and eye bath.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator.
Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES
Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance — Physical State: Liquid

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.
Ingestion: May be harmful if swallowed.

SIGNS AND SYMPTOMS OF EXPOSURE
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA
Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Irritant.
Risk Statements: Irritating to eyes, respiratory system and skin.
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
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