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
ADDRESS	4011 Dental Sciences Building, University of Western Ontario
PHONE NUMBER	519-661-2111 Ext 81562
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 (both healthy volunteers and diabetic patients, LHSC/SJHC, Ethics Approval 14050), human bone marrow (commercial, please see below), and human tumour specimens (LHSC, Ethics Approval 14047E) 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 (aortic and brain-derived) 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 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 (please see appendices attached). These 3rd generation biosafety plasmids will be used only for stable transfection of our primary cells. We will not use the plasmids for stock preparation. The target genes for our studies are insulin-like growth factors and platelet-derived growth factors (and corresponding receptors, see appendix). 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
Human cells (skin, umbilical cord)	Commercial	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 may 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	O Yes ☑ No		
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 ☑ 2 O 2+ O 3

*** a list of cells used in the laboratory is presented in Appendix A.

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	**1. Blood**/Commercial (US Biological, Stem Cell Tech, Allcells, Lonza, 3HBiomedical, and Promo Cell GmbH) **2. Blood**/Healthy and diabetic patient blood (LHSC, ethics approval 14050)	O Yes ☑ Unknown		O 1 ☑ 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	LHSC, tumour specimens	O Yes ☑ Unknown		O 1 ☑ 2 O 2+ O 3
Human Organs or Tissues (preserved)	Fixed tissue slides (Abcam, Ray Biotech, Imgenex Corp) and fixed tissue blocks (US Biomax)	Not Applicable		Not Applicable

*** a list of cells (from blood and tissue specimens) used in the laboratory is presented in Appendix A.

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 (these plasmids are ready to be used for transfections and will not be used for produce virus)*	*Santa Cruz Biotech*	*A number of genes will be targeted including insulin-like growth factors and platelet-derived growth factors*	*Transfection is expected to change the differentiation capacity of adult stem cells.*

* 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				

See E-mail

* 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

*** a list of shRNA used in the laboratory is presented in Appendix B.

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 _____ ZIA A. KHAN Date: 02/02/2011

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:

E-mail

----- Original Message --------
Subject: Re: Biological Agents Registry Form (Khan)
Date: Wed, 02 Feb 2011 17:07:39 -0500
From: Zia Khan <Zia.Khan@schulich.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Thanks Jennifer.
I am somewhat confused about this section. I do plan to use viral vectors that express shRNA in mammalian cells. But these vectors will not be used for virus production and we will not be 'transducing'. I would go ahead with a no to the question 4.3.

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

APPENDIX A: Cells

1. List of cells and sources.
2. Information sheets on Bovine cells

Appendix A: List of cells and sources.

HUMAN CELLS	Source	Catalogue #
Human Umbilical Vein Endothelial Cells	Lonza	CC-2517
Human Neonatal Dermal Microvascular Endothelial Cells	Lonza	CC-2516
Human Mixed Astrocytes	Lonza	CC-2565
Human Umbilical Cord Endothelial Colony Cells	Lonza	189423
Human Bone Marrow Mononuclear Cells	Lonza	2M-125B
Human Bone Marrow Mesenchymal Stem Cells	Lonza	PT-2501
Human Peripheral Blood Mononuclear Cells	Lonza	CC-2702
Human Umbilical Arttery Smooth Muscle Cells	Lonza	CC-2579
Human Neonatal Dermal Fibroblasts	Lonza	CC-2509
Human Whole Blood Cells	All Cells	WB002
Human Whole Blood Cells	US Biologicals	B2150-01-50ML
Human Peripheral Blood Mononuclear Cells (*Healthy)	Volunteers	Ethics Approval 14050
Human Peripheral Blood Mononuclear Cells (*Diabetic)	Patients	Ethics Approval 14050
Human Infantile Hemangioma Cells	Patients	Ethics Approval 14047E

OTHER CELLS***	Source	Catalogue #
Bovine Aortic Endothelial Cells	Lonza	BW-6002

***Bovine Cells:**

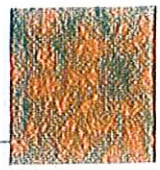
We would like to use these bovine cells to study the contribution of different cell types to blood vessel formation. Specifically, we would like to co-culture these cells with human cells. We would then label endothelial cells with species-specific antibodies.

These bovine cells are:

1. Available commercially.
2. The animals have been USDA inspected.
3. The cells are proliferating (usually shipped at passage >2).

We do not expect these cells to be of any greater risk than other cells that are in culture.

Lonza Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Document # CC-24-4 01/08
Walkersville, MD 21793-0127 USA



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	***	***	P305
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 tissue 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 [illegible] has been reviewed by Quality Assurance for compliance with requirements of Lonza's Quality System.
The [illegible] was prepared from a validated Part 11 compliant electronic system and bears [illegible] signature in lieu of signature.

-------- 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

APPENDIX B: Plasmids and ShRNA

1. List of shRNA plasmids.
2. Information sheets on the plasmids.

Appendix B. ShRNA for gene knockdown.

Plasmids***	Source	Catalogue #
PDGFR-ß shRNA Plasmid (h)	Santa Cruz Biotechnology	sc-29442-SH
PDGFR-a shRNA Plasmid (h)	Santa Cruz Biotechnology	sc-29443-SH
IGF-II shRNA Plasmid (human)	Santa Cruz Biotechnology	sc-39576-SH
Control shRNA Plasmid A	Santa Cruz Biotechnology	sc-108060
IGF-IRa shRNA Plasmid (h)	Santa Cruz Biotechnology	sc-29358-SH
IGF-IIR shRNA Plasmid (h	Santa Cruz Biotechnology	sc-37118-SH

***Plasmids and Transfections:**

The plasmids that we plan to use are 3rd generation plasmids. These will be used to transfect the human cells (appendix A) and not for virus preparation or cell infection. Please see attached email correspondence with Santa Cruz representative detailing the plasmids. In addition to the plasmids listed here, we may include more shRNA-plasmids with the same format and from the same vendor.

We do not expect these plasmids to be of health risk.

From: Zia Khan
To: jstanle2@uwo.ca
Date: 16/07/2009 4:21 pm
Subject: Fwd: Santa Cruz Biotechnology
Attachments: Request for BSL2.doc

Hi Jennifer,
I am attaching our request to stay as BSL2. I am also forwarding the email I have received from Santa Cruz Biotech stating that the plasmid system is a new generation system with packaging and vector plasmids separated (multiple plasmid system). Therefore, there is no way we can produce virus particles without the other plasmids - we do not intent to do that.
Thank you so much for all your help.
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

>>> Stacy Haff <haffs@scbt.com> 16/07/2009 3:44 pm >>>

Dear Dr.,

To answer your questions, I have provided the following regarding our shRNA plasmid and shRNA lentiviral particles:

1) SCBT's Lentiviral Particles are produced in 293T cells with the backbone vector plasmid and 3 helper plasmids. (3rd generation biosafety)
2) The genes for gag/pol are on a different plasmid than the gene for rev.
3) The Lentiviral Particles are VSV-G pseudotyped, with VSV-G as the envelope protein.
4) There are no other genes in addition to gag, pol, rev, and env are present on the plasmids in the packaging kit mix?
- The promoter in the lentivector allows efficient Tat-independent production of viral RNA, reducing the number of genes from HIV that are used in this system.
- The number of lentiviral genes necessary for packaging, replication and transduction is reduced to three. The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, the expression vector, or any other vector, to prevent generation of recombinant replication-competent virus.
- None of the HIV genes will be present in the packaged viral genome. This is because they are expressed from the packaging vector which lacks a packaging signal, thereby creating replication-incompetent lentiviral particles.

Please feel free to contact me with any further questions or concerns.

Sincerely,

Stacy Haff
Technical Service Representative
Santa Cruz Biotechnology, Inc.
stacy@scbt.com
800.457.3801 ext 113

SANTA CRUZ BIOTECHNOLOGY, INC.

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	Delivers shRNA Plasmid DNA into cells with minimal cell toxicity, enables highly efficient shRNA Plasmid DNA transfection in a variety of cell lines including CHO-K1, COS, LNCaP, NIH/3T3, 293, T24, C2C12, SF-9, primary human keratiocytes, primary aortic smoothmuscle, primary rabbit myoblasts, human bone marrow endothelial cells (HBMEC).	0.2 ml 50-100 transfections
shRNA Plasmid Transfection Medium	sc-108062	Reduced-serum medium suitable for addition to shRNA suspension and shRNA Transfection Reagent immediately prior to cell transfection, modification of Eagle's Minimal Essential Medium, buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors and phenol red.	20 ml
Control shRNA Plasmid-A	sc-108060	Control shRNA Plasmid-A is a negative control for experiments using targeted shRNA transfection which encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA.	20 µg 20 transfections
Control shRNA Plasmid-B	sc-108065	Control shRNA Plasmid-B is available as an alternate negative scrambled shRNA sequence control.	20 µg 20 transfections
Control shRNA Plasmid-C	sc-108066	Control shRNA Plasmid-C is available as an alternate negative scrambled shRNA sequence control.	20 µg 20 transfections

shRNA Plasmid support reagents are optimal for successful delivery of Santa Cruz Biotechnology, Inc.'s shRNA Gene Silencing Plasmids into mammalian cells. Amounts listed above are based on use of 6-well plates.

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. Raizis, 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 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-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: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use 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 detailed protocols and support products.

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-I 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 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-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-TR: 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, 537 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-IIR shRNA Plasmid (h): sc-37118-SH

BACKGROUND

The mannose 6-phosphate/insulin-like growth factor II receptor, IGF-IIR (also designated M6P/IGF2R), is an ubiquitously expressed integral glycoprotein. By binding glycoproteins through two of its extracytoplasmic domains, IGF-IIR mediates the activation of TGFb1 (a growth inhibitor), the degradation of IGF-II and the transport of lysosomal enzymes. Subsequently, IGF-IIR can form oligomeric complexes, which increase the affinity of IGF-IIR for lysosomal enzymes. Unlike IGF-IR, IGF-IIR does not potentiate the signaling of IGF-I or IGF-II, which have mitogenic, cell survival and insulin-like effects. Therefore, IGF-IIR is characterized as a tumor suppressor. Furthermore, the IGF-IIR gene is located on chromosome 6q26, which is commonly mutated or deleted in several human cancers.

REFERENCES

1. Ellis, M.J., et al. 1998. Insulin-like growth factors in human breast cancer. Breast Cancer Res. Treat. 52: 175-184.
2. Braulke, T. 1999. Type-2 IGF receptor: a multi-ligand binding protein. Horm. Metab. Res. 31: 242-246.
3. Lorenzo, K., et al. 2000. Invasive properties of murine squamous carcinoma cells: secretion of matrix-degrading cathepsins is attributable to a deficiency in the mannose 6-phosphate/insulin-like growth factor II receptor. Cancer Res. 60: 4070-4076.
4. Gemma, A., et al. 2000. Mutation analysis of the gene encoding the human mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) in human cell lines resistant to growth inhibition by transforming growth factor β1 (TGFβ1). Lung Cancer 30: 91-98.

CHROMOSOMAL LOCATION

Genetic locus: IGF2R (human) mapping to 6q26.

PRODUCT

IGF-IIR 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 plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see IGF-IIR siRNA (h): sc-37118 and IGF-IIR shRNA (h) Lentiviral Particles: sc-37118-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-IIR shRNA Plasmid (h) is recommended for the inhibition of IGF-IIR 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-IIR (C-15): sc-14410 is recommended as a control antibody for monitoring of IGF-IIR 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: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use 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-IIR gene expression knockdown using RT-PCR Primer: IGF-IIR (h)-PR: sc-37118-PR (20 µl, 464 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.

PDGFR-α shRNA Plasmid (h): sc-29443-SH

BACKGROUND

Platelet-derived growth factor (PDGF) is a mitogen for mesenchyme- and glia-derived cells. PDGF consists of two chains, A and B, which dimerize to form functionally distinct isoforms PGDF-AA, PDGF-AB and PDGF-BB. These three isoforms bind with different affinities to two receptor types, PDGFR-α and -β, which are endowed with protein tyrosine kinase domains. PDGFR-α can bind to both A and B subunits of PDGF, while PDGFR-β can only bind the B subunit. Ligand binding promotes either homo- or heterodimerization of the PDGF receptors in a specific manner. PDGF-AA induces the dimerization of two α receptors, PDGF-AB induces dimerization of αα and αβ, and PDGF-BB induces the formation of three types of dimers: αα, αβ and ββ. The genes encoding PDGFR-α and -β map to human chromosome 4q12 and 5q33.1, respectively. Translocation of the PDGFR-β gene with the TEL gene is linked with chronic myelomonocytic leukemia (CMML), a myelodysplastic syndrome, and demonstrates the oncogenic potential of the PDGF receptors.

REFERENCES

1. Ross, R., et al. 1986. The biology of platelet-derived growth factor. Cell 46: 155-169.
2. Hart, C.E., et al. 1988. Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240: 1529-1531.
3. Heldin, C., et al. 1988. Binding of different dimeric forms of PDGF to human fibroblasts: evidence for two separate receptor types. EMBO J. 7: 1387-1393.
4. Rupp, E., et al. 1994. A unique autophosphorylation site in the plaelet-derived growth factor α receptor from a heterodimeric receptor complex. Eur. J. Biochem. 225: 29-41.
5. Bazenet, C.E., et al. 1996. Phosphorylation of tyrosine 720 in the platelet-derived growth factor α receptor is required for binding of GRB2 and SHP-2 but not for activation of Ras or cell proliferation. Mol. Cell. Biol. 16: 6926-6936.

CHROMOSOMAL LOCATION

Genetic locus: PDGFRA (human) mapping to 4q12.

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.

PROTOCOLS

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

PRODUCT

PDGFR-α 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 PDGFR-α siRNA (h): sc-29443 and PDGFR-α shRNA (h) Lentiviral Particles: sc-29443-V as alternate gene silencing products.

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

PDGFR-α shRNA Plasmid (h) is recommended for the inhibition of PDGFR-α 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

PDGFR-α (C-20): sc-338 is recommended as a control antibody for monitoring of PDGFR-α 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-TR: 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 PDGFR-α gene expression knockdown using RT-PCR Primer: PDGFR-α (h)-PR: sc-29443-PR (20 µl, 500 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SANTA CRUZ BIOTECHNOLOGY, INC.

PDGFR-β shRNA Plasmid (h): sc-29442-SH

BACKGROUND

Platelet-derived growth factor (PDGF) is a mitogen for mesenchyme- and glia-derived cells. PDGF consists of two chains, A and B, which dimerize to form functionally distinct isoforms, PDGF-AA, PDGF-AB and PDGF-BB. These three isoforms bind with different affinities to two receptor types, PDGFR-α and -β, which are endowed with protein tyrosine kinase domains. PDGFR-α can bind to both A and B subunits of PDGF, while PDGFR-β can only bind the B subunit. Ligand binding promotes either homo- or heterodimerization of the PDGF receptors in a specific manner. PDGF-AA induces the dimerization of two α receptors, PDGF-AB induces dimerization of αα and αβ and PDGF-BB induces the formation of three types of dimers, αα, αβ and ββ. Translocation of the PDGFR-β gene with the Tel gene is linked to chronic myelomonocytic leukemia (CMML), a myelodysplastic syndrome, and demonstrates the oncogenic potential of the PDGF receptors.

REFERENCES

1. Ross, R., et al. 1986. The biology of platelet-derived growth factor. Cell 46: 155-169.
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CHROMOSOMAL LOCATION

Genetic locus: PDGFRB (human) mapping to 5q33.1.

PRODUCT

PDGFR-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 plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see PDGFR-β siRNA (h): sc-29442 and PDGFR-β shRNA (h) Lentiviral Particles: sc-29442-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

PDGFR-β shRNA Plasmid (h) is recommended for the inhibition of PDGFR-β 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

PDGFR-β (P-20): sc-339 is recommended as a control antibody for monitoring of PDGFR-β 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-TR: 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 PDGFR-β gene expression knockdown using RT-PCR Primer: PDGFR-β (h)-PR: sc-29442-PR (20 µl, 423 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-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

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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/β (h)-PR: sc-29358-PR (20 µl, 504 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.