Modification Form for Permit BIO-UWO-0191

Permit Holder: Zia Khan

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

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	human (primary), blood, tumour, Rodent (primary) blood, tissues	Bovine Aortic Endothelial Cells
Approved Use of Human Source Material	blood (whole), blood (fraction) mononuclear cells, tissues (unpreserved) hemangioma specimens	
Approved GMO	siRNA or plasmid transfection agent	
Approved use of Animals	nu/nu mice, B6 mice	
Approved Toxin(s)		

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 7, 2008

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

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.*

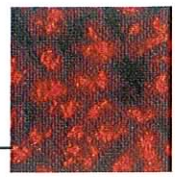
[2] *Blood samples from diabetic patients will be obtained through collaboration with Dr. Jeffrey L. Mahon (LHSC/SJHC)*

[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.

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

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: BW-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 in 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.

Modification Form for Permit BIO-UWO-0191

Permit Holder: Zia Khan

Approved Personnel (Please stroke out any personnel to be removed)	Additional Personnel (Please list additional personnel here)
	1. Alexandra Kleiman, MSc candidate

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	human (primary), blood, tumour, Rodent (primary) blood, tissues	
Approved Use of Human Source Material	blood (whole), blood (fraction) mononuclear cells, tissues (unpreserved) hemangioma specimens	
Approved GMO	siRNA or plasmid transfection agent	1. lentiviral plasmid (for shRNA delivery; commercially available) 2. pCMV plasmid (for cDNA delivery; commercially available)
Approved use of Animals	nu/nu mice, B6 mice	
Approved Toxin(s)		

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 7, 2008

Signature of Permit Holder:

BioSafety Officer(s): July 17/09

Chair, Biohazards Subcommittee:

* level 2 adequate (no virus production - see attached). If (in the future) virus will be produced, project will need to be assessed.

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 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-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 preproinsulin-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.

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 keratinocytes, primary aortic smooth muscle, 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 Plasmid 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 optimized for successful delivery of Santa Cruz Biotechnology, Inc.'s shRNA Gene Silencing Plasmids into mammalian cells. Amounts listed above are based on use of 3-well plates.

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
SC320234	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 Add to Shopping Cart

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. 2004. 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: 5161-5174.
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. Hayashi, K., et al. 2004. Insulin receptor substrate-1/SHP-2 interaction, a phenotype-dependent switching machinery of Insulin-like growth factor-I signaling in vascular smooth muscle cells. J. Biol. Chem. 279: 40807-40818.

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.

LIST OF ATTACHMENTS

1. **Description of Experiments**
2. **MSDS – cDNA Clones**[i]
3. **Description – pCMV Vector**[ii]
4. **MSDS – Lentiviral Plasmids**[iii]

---

[i] cDNA of choice inserted in the pCMV6-AC vector is available commercially from Origene.

[ii] Description of the pCMV6-AC vector. We were unable to find MSDS for the vector.

[iii] shRNA against gene of interest is available in the lentiviral plasmids.

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*. These techniques 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 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 are available commercially). 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 (also commercially available). 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

[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 blood or tumour specimens) in athymic nu/nu mice. Briefly, cells will be resuspended in Matrigel (BD Biosciences; solubilised extracellular matrix preparation) 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.

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 H_2O: 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
The information contained in this MSDS was obtained from sources we believe are reliable. However, the above information is provided without warranty, expressed or implied, regarding its correctness. *OriGene makes no guarantee of the accuracy or completeness of the data and shall not be liable for any damages thereto. The data are offered solely for your consideration, investigation, and verification. These suggestions should not be confused with state, municipal, or insurance requirements, or with national safety codes and constitute no warranty. The conditions or methods if handling, storage, use and disposal of the product are beyond our control and may be beyond our knowledge.* ***Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution.*** *Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.* ***Any use of these data and information must be determined by the user to be in accordance with applicable federal, state, and local regulations.***

N/A - Not applicable or no information available.

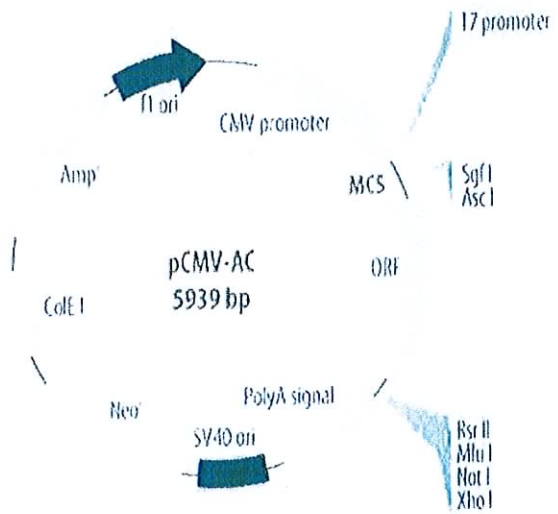
pCMV6-AC Non-Tagged PrecisionShuttle Vector

Specifications | Custom Cloning | Product Manual | FAQs

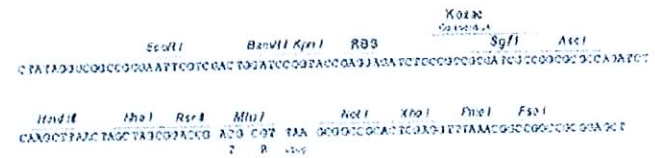
Catalog No.	Description	Price
PS100020	PrecisionShuttle pCMV6-A (non-tagged) Destination Vector	$320

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 RsrII 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 RsrII/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



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
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice

B10-UWO-0191

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR* Zia A. Khan
SIGNATURE _____
DEPARTMENT Pathology
ADDRESS 4011 Dental Sciences Build., 1151 Richmond Street
PHONE NUMBER 519-661-2111 Ext 81562
EMAIL zia.khan@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 4004, 4011, 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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
Stem cells in vascular repair and homeostasis (PI Startup)
Role of vascular stem cells in diabetic complications (Applied - HSFC Grant)
Mechanism of endothelial differentiation in hemangioma vasculogenesis (Applied CIHR & NCIC Grants)

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES PI Startup, HSFC, CIHR, NCIC

Names of all personnel working under Principal Investigators supervision in this location:
i) N/A**
ii) _____
iii) _____
iv) _____
v) _____

** All personnel hired will be required to attend the following workshops:
a) Employee health and safety orientation
b) laboratory and environmental/waste management workshop
c) Biosafety

In addition, WHMIS training will be required.

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

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required. 1 2 3

1.4 Source of microrganism(s) or biological agent(s)? _____

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?		Source of Primary Cell Culture Tissue
Human	☒ Yes	☐ No	human blood & human tumour specimens
Rodent	☒ Yes	☐ No	mouse blood and tissues
Non-human primate	☐ Yes	☒ No	
Other (specify)			

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

Cell Type	Is this cell type used in your work?		Specific cell line(s)	Supplier / Source
Human	☐ Yes	☒ No		
Rodent	☐ Yes	☒ No		
Non-human primate	☐ Yes	☒ No		
Other (specify)	☐ Yes	☐ No		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 [2] 3

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

3.0 Use of Human Source Materials

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

3.2 Indicate if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☐ YES ☐ NO If YES, Specify Whole blood for cell isolation
- Human blood (fraction) or other bodily fluids ☒ YES ☐ NO If YES, Specify mononuclear cells from blood
- Human organs (unpreserved) ☐ YES ☐ NO If YES, Specify _____
- Human tissues (unpreserved) ☒ YES ☐ NO If YES, Specify human hemangioma specimens

3.3 Is human source known to be infected with and infectious agent ☐ YES ☒ NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

4.0 Genetically Modified Organisms and Cell lines

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

4.2 Will genetic sequences from the following be involved:

- HIV ☐ YES ☐ NO
if YES specify _____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐ YES ☐ NO
if YES specify _____
- Other human or animal pathogen and or their toxins ☐ YES ☐ NO
if YES specify _____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☐ YES ☐ NO If YES specify _____
- Known oncogenes ☐ YES ☐ NO If YES specify _____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES ☐ NO
If YES name virus _____

4.5 List specific vector(s) to be used _____

4.6 Will virus be replication defective ☐ YES ☐ NO

4.7 Will virus be infectious to humans or animals ☐ YES ☐ NO

4.8 Will this be expected to increase the Containment Level required ☐ YES ☐ NO

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

5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☒ YES ☐ NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used Experiments will bo performed on athymic nu/nu mice and B6 mice

6.3 AUS protocol # The protocol for animal care and use will be submitted by the PI

6.4 If using murine cell lines, have they been tested for murine pathogens? ☐ YES ☐ NO ☒ N/A

7.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs ☐ YES ☐ NO
- Pound source cats ☐ YES ☐ NO
- Sheep or goats ☐ YES ☐ NO
- Non- Human Primates ☐ YES ☐ NO If YES specify species _____
- Wild caught animals ☐ YES ☐ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin _____

8.3 What is the LD_{50} (specify species) of the toxin _____

9.0 Import Requirements

9.1 Will the agent be imported? ☐ YES ☒ NO
If no, please proceed to Section 10.0
If yes, country of origin _____

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

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

9.4 Has the import permit been sent to OHS? ☐ YES ☐ NO
If yes, Permit # _____

10.0 Training Requirements for Personnel named on Form

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

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

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

SIGNATURE _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 [2] 3

11.2 Has the facility been certified by OHS for this level of containment? ☐ YES ☒ NO

11.3 If yes, please give the date and permit number: The inspection will be scheduled after purchasing equipment for the laboratory

12.0 Approvals

UWO Biohazard Subcommittee

Signature _____ Date 7 Jan '08

Safety Officer for Institution where experiments will take place:

Signature _____ Date _____

Safety Officer for University of Western Ontario (if different than above)

Signature Jennifer Stanley Date Jan. 4/08

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

DESCRIPTION OF EXPERIMENTS CONDUCTED IN Dr. KHAN'S LABORATORY.

Location: Rooms 4004, 4011, and 4020 Dental Sciences Building.
Brief Description: Dr. Khan's 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 tumor 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*. These techniques 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. Finally, cells are injected in athymic nude mice using matrix substrate (Matrigel; BD Biosciences) to study the behaviour in an *in vivo* setting.

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	Application
	Diabetic Patients[1]	Application
Human blood/bone marrow mononuclear cells	Commercial	N/A
Human tissue	Hemangioma patients[2]	Application
Mouse blood	Nu/nu mice	Application
Explanted mouse tissue	Nu/nu & B6 mice	Application

[1] *Blood samples from healthy volunteers will be collected upon approval of REB.*
[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 Dr. Nancy Chan (Pathology/LHSC)*

Animal Experiments: Dr. Khan will investigate the function of primary cells (isolated from blood or tumour specimens) in athymic nu/nu mice. Briefly, cells will be resuspended in Matrigel (BD Biosciences, solubilised extracellular matrix preparation) 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. Dr. Khan is in the process of applying for the protocol for animal use and care.

For further information, please contact Dr. Khan.

Zia A. Khan, PhD
Assistant Professor
Department of Pathology
4011 – Dental Sciences Building
University of Western Ontario
London, ON N6A 5C1
Tel (519) 661-211 Ext 81562
Fax (519) 661-3370
Email: zia.khan@schulich.uwo.ca

Subject: Re: Biohazard form - KHAN
From: Zia Khan <Zia.Khan@schulich.uwo.ca>
Date: Wed, 26 Dec 2007 11:07:25 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

We have two major projects in which we are trying to identify the molecular basis of two diseases. Just to give you a brief description - once we identify genes which exhibit altered expression (downregulation or upregulation in the context of the disease), we will target these genes in the cells isolated from the patients (HSREB applications to be submitted Jan 02) by either gene transfection (transfection ready plasmids are commercially available and/or can be custom made) or siRNA gene knockdown (again, commercially available). We do not plan to carry out plasmid prep in the lab (no E. coli/no packaging cell line). For siRNA, there is no expression vector - these are small RNA molecules which readily pass the cell membrane by simple transfection reagent (e.g. lipofectamine). For gene transfections, the genes of interest are already packaged in expression vectors in a ready-to-transfect formulation. Cells will be cultured for 0-12 hours in the presence of siRNA or plasmid containing the gene of interest + transfection reagent (Lipofectamine) ([illegible] from Ambion/[illegible]).

If you have any questions, please email/call.
Thanks
Zia

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 ex: 81562
Fax: (519) 661-3797

[illegible] <jstanle2@uwo.ca> [illegible]