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): _____

Chair,Biohazards Subcommittee: _____

LIST OF ATTACHMENTS

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

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[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

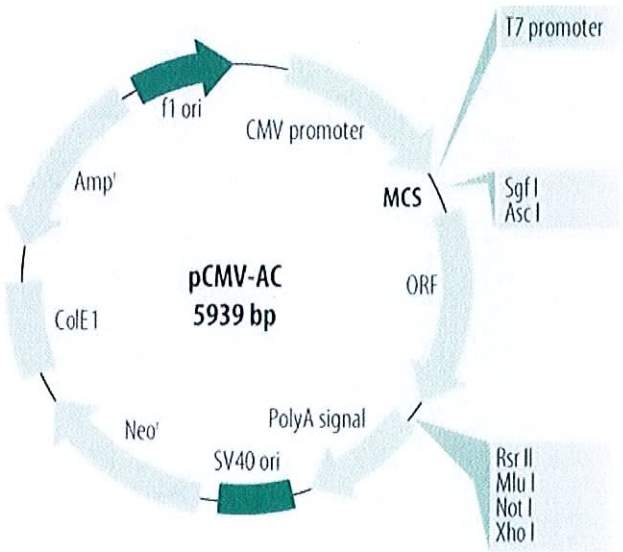
Specifications | Custom Cloning | Product Manual | FAQs

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

Add to Shopping Cart

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 Pme I, then it will express Myc-DDK tagged protein.



pCMV6-A

Kozac Consensus

EcoR I BamH I Kpn I RBS Sgf I Asc I

CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

Hind III Nhe I Rsr II Mlu I Not I Xho I Pme I Fse I

CAAGCTTAACTAGCTAGCGGACCG ACG CGT TAA GCGGCCGCACTCGAGGTTTAAACGGCCGGCCGCGGAGCT

T R Stop

Schematic of the multiple cloning sites:

Download full vector sequence

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, WHIMIS 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)? ف YES ف 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?
	ف Yes ف No	ف Yes ف No	ف Yes ف No	
	ف Yes ف No	ف Yes ف No	ف Yes ف No	
	ف Yes ف No	ف Yes ف No	ف Yes ف No	
	ف Yes ف No	ف Yes ف No	ف Yes ف 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? ف YES ف 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	ف Yes ف No	human blood & human tumour specimens
Rodent	ف Yes ف No	mouse blood and tissues
Non-human primate	ف Yes ف 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	ف Yes ف No		
Rodent	ف Yes ف No		
Non-human primate	ف Yes ف No		
Other (specify)	ف Yes ف 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

→ siRNA or plasmid + transfection agent
(Sel attached)

* 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? ف YES [ف 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? ف YES ف 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? ف YES ف NO

6.0 Animal Experiments

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

6.2 Name of animal species to be used Experiments will be 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? ف YES ف 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 ف YES [ف NO]
- Pound source cats ف YES [ف NO]
- Sheep or goats ف YES [ف NO]
- Non- Human Primates ف YES [ف NO] If YES specify species _____
- Wild caught animals ف YES [ف NO] If YES specify species _____
  colony # _____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ف YES [ف 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_____

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

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 G M. Kidder 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.

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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
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4011 – Dental Sciences Building
University of Western Ontario
London, ON N6A 5C1
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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 4-12 hours in the presence of siRNA or plasmid containing the gene of interest + transfection reagent (lipofectamine, LiSport from Ambion etc...).

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

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Jennifer Stanley <jstanle2@uwo.ca> 21/12/2007 4:22 pm >>>
Hello Dr. Khan
It was nice meeting you the other day.
In Section 4.0 - you have said that you do not do any genetic modifications...Can you explain the "gene over-expression and knockdown"
that you do and how this is accomplished?
Thanks!
Happy holidays
Jennifer