Modification Form for Permit BIO-UWO-0171

Permit Holder: Morris Karmazyn

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

Siichi Taniai

Nazo Said

Cathy Huang

Eduardo Martinez

Melissa Moey

Jim Haist

Tracey Gan

Venkatesh Rajapurohitam

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms		E Coli K12 JM101 HEK-293 Cells
Approved Primary and Established Cells	[primary]: rodent heart. [established]: cardiomyocytes	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)		pFLAG-CMV4 pAdTrack-CMV IP-eGFP Adenovirus T7 pSilencer tM adeno 1.0-CMV system
Approved Use of Animals	Sprague Dawley Rat (2009-020)	
Approved Biological Toxin(s)		

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

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

Signature of Permit Holder: _____

Current Classification: 1 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: Dec 23, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

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

-------- Original Message --------
Subject: Re: Karmazyn Modification
Date: Tue, 03 Jan 2012 11:18:29 -0500
From: Tracey Gan <tgan@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC:morris.karmazyn@schulich.uwo.ca

Hi Jennifer

Please see the following details and the attached files as your requested in your email on Dec 21, 2011. Let me know if there are any further questions

There is no specification on which genes will be used in the GMO section.

COX-2 gene will be used for the siRNA transfection (non-virus): details are described in the experimental procedures under the siRNA transfection.

CK2a gene will be used for the adenovirus infection using the pSilencer adeno 1.0-CMV system system. Details are described in the experimental prcedure under the constructions of adenoviruses Harboring target iRNA and adenoviurs infection.

On page 2 there is no containment level listed for added biohazard(s).
the containment level is 2.

The form does not provide enough information to understand what will be done.
We provided a one page summary as you requested. Please see the reattached decription of the experimental procedures.

The form is difficult to read and should be submitted electronically instead.
I have typed the words and scaned the form and resubmitted it via email as requested.

E Coli K12 JM101 will be used to amplify the plasmids/vectors: See attached 1

HEK-293 Cells will be used to amplify the virus: See attached 2

pFLAG-CMV4:see attached 3

pAdTrack-CMV:see attached 4

IP-eGFP Adenovirus:See attached 5

T7:
T7 is included in the *Silencer*® siRNA Construction Kit (with manual) and can be purshased from Applied Biosystems (Ambion) which belongs to Life Techaniques now. the Cat#: AM1620M. the
web: https://products.invitrogen.com/ivgn/product/AM1620M?ICID=search-product.

pSilencer tM adeno 1.0-CMV system: pSilencer adeno 1.0-CMV system can be purshased from Applied Biosystems (Ambion) which belongs to Life Techaniques now.
the cat #: AM5790.
the web: https://www.invitrogen.com/search/global/searchAction.action?resultPage=3&resultsPerPage=15&query=Silebcer+siRNA+construction+kit.

If you require any additional specific information please let me know.

Regards, Tracey

Tracey Gan, MSc
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On 12/22/11, **Jennifer Stanley** <jstanle2@uwo.ca> wrote:

Hi there
Here are the (draft) notes from the meeting. Please edit and resend so it can be approved in January:

> There is no specification on which genes will be used in the GMO section. On page 2 there is no containment level listed for added biohazard(s). The form does not provide enough information to understand what will be done. The form is difficult to read and should be submitted electronically instead.
>
> Regards
> Jennifer

FAVORITE TOOLS

- Enzyme Finder
- NEBcutter
- NEBuffer Chart
- Double Digest Finder
- Isoschizomers
- DNA Sequences and Maps
- PCR Selection
- Tm Calculator
- REBASE

SPECIAL OFFERS

E. coli K12 JM101

Limit of 1 per order

Catalog #	Size	Concentration	Price	Qty	
E4106S	200 µl		$0.00	1	ADD TO CART

Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

Description:
A suspension of *E. coli* K12 JM101 which has been grown in minimal media and brought to 50% glycerol.

Genotype: F′ *traD36 proA*$^+$*B*$^+$ *lacI*^{q} Δ*(lacZ)M15/* Δ*(lac-proAB) glnV thi*

Recommended Growth Medium: M9
Growth Temperature: 37°C

Strain Properties

dam/dcm Methylation: dam$^+$, dcm$^+$
Protease-deficient: No
LacI^q: Yes

Storage Conditions

Storage Temperature:
-20°C
For long term storage (>30 days), store at -70°C.

Notes

Usage notes:
1. Rubidium Chloride Method: RbCl Transformation Protocol

References

1. Messing, J. (1979) A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. *Recomb. DNA Tech. Bull. (NIH)*, 3(2), 43-48.
2. Yanish-Perron, C. et al. (1985) *Gene*, 33, 103-119.

HEK-293 cells

Cell Biology

ATCC® Number:	**CRL-1573™**	Order this Item	Price:	**$279.00**

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing transfection host viruscide testing
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required ▸

Product Information Sheet

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management: basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

pFLAG-CMV™-4 Expression Vector

Catalog Number **E7158**
Storage Temperature –20 °C

Product Description

pFLAG-CMV-4 is a 6271 bp expression vector used for transient or stable expression in mammalian cells. The vector is a derivative of pCMV5[1] used for transient or stable expression of a properly inserted open reading frame as an N-terminal Met-FLAG® fusion protein.

The promoter-regulatory region of the human cytomegalovirus[2] drives transcription of FLAG-fusion constructs. The aminoglycoside phosphotransferase II gene[3] (neo^r) confers resistance to aminoglycosides such as G 418,[4] allowing for selection of stable transfectants.

The pFLAG-CMV-4 Expression Vector is a shuttle vector containing both bacterial and SV40 origins of replication for propagation in both *Escherichia coli* and mammalian cells. Efficiency of replication and genomic integration is optimal when using host cells that express the SV40 large T antigen (e.g. COS-7).

The FLAG epitope is a small, hydrophilic 8 amino acid tag (DYKDDDDK)[5] that provides for sensitive detection and high quality purification using ANTI-FLAG® products (visit www.sigma-aldrich.com for a complete listing). Removal of the N-terminal FLAG tag is possible using enterokinase, which cleaves following the Asp-Asp-Asp-Asp-Lys recognition site at the C-terminal end of the FLAG peptide.

pFLAG-CMV-4-BAP Control Plasmid is a 7.7 kb derivative of pCMV5[1] used for transient intracellular expression of N-terminal Met-FLAG® bacterial alkaline phosphatase fusion protein in mammalian cells.

The promoter-regulatory region of the human cytomegalovirus[2] drives transcription of bacterial alkaline phosphatase. The amino glycoside phospho-transferase II gene[3] (Neo) confers resistance to aminoglycosides such as G 418.[4]

pFLAG-CMV-4-BAP Control Plasmid is a shuttle vector for *E. coli* and mammalian cells. Efficiency of replication and genomic integration is optimal when using an SV40 T antigen-expressing host, such as COS cells.

Map positions of key features in the pFLAG-CMV-4 Expression Vector and the pFLAG-CMV-4-BAP Control Plasmid can be found at www.sigma.com/vectormaps .

Sequence verification of the MCS can be performed using the N-CMV-30, Catalog Number P5350, and C-CMV-24, Catalog Number P5475, Sequencing Primers.

Components

- pFLAG-CMV-4 Expression Vector 20 µg
  Catalog Number E1775
  Supplied as 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

- pFLAG-CMV-4-BAP Control Plasmid 20 µg
  Catalog Number C4722
  Supplied as 0.5 mg/ml in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA,.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C

References

1. Andersson, S., *et al., J. Biol. Chem.*, **264**, 8222-8229 (1989)
2. Thomsen, D.R., *et al.,* Proc. Natl. Acad. Sci. USA, **81**, 659-663 (1984)

addgene
A better way to share plasmids

Browse > Bert Vogelstein > He et al > pAdTrack-CMV

Plasmid 16405: pAdTrack-CMV

Gene/insert name:	None
Vector backbone:	pAdTrack-CMV (Search Vector Database)
Vector type:	Mammalian Expression, Adenoviral
Backbone size w/o insert:	9220
5' sequencing primer:	n/a List of Sequencing Primers
Bacterial resistance:	Kanamycin
Growth strain:	DH10B
Growth temperature (℃):	37
High or low copy:	Low Copy
Sequence:	View sequences (3)
Map:	View map
Supplemental document:	AdEasy Protocol (application/pdf)
Principal Investigator:	Bert Vogelstein
Terms and Licenses:	MTA

Comments: Vector pAdTrack-CMV is used for expression of transgenes under a CMV promoter when a GFP tracer is desired.

Max insert size = 5.0kb with pAdEasy1 and 7.7kb with pAdEasy2.

See attached protocol and http://www.coloncancer.org/adeasy.htm for more information.

Note that there are discrepancies between Addgene's quality control sequence and the assembled sequenced from the depositing lab. These differences are not known to affect the function of the plasmid.

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

Applied Biological Materials Inc.
Telephone: 1-866-757-2414
Email: info@abmgood.com
Website: www.abmGood.com

IP-eGFP Adenovirus

Cat. No.	Quantity	Titer	Accession Number
000066A	250µl	1x10^{6}pfu/mL	

Vector	pAdeno
Promoter	CMV
Insert	IP-eGFP
Organism	Human
Gene Type	Wild Type
Titer	1x10^{6}pfu/mL
Storage	DMEM with 2.5% glycerol

EXPERIMENTAL PROCEDURES:

Cardiomyocytes Isolation and Culture

Cardiomyocyte are isolated from 1- to 2-day-old Sprague-Dawley rat heart ventricles as described previously (Karmazyn et al., 2003). Briefly, after dissection hearts are washed and minced in HEPES-buffered saline solution (containing (mm): 130 NaCl, 3 KCl, 1 NaH2PO4, 4 glucose, and 20 HEPES; pH adjusted to 7.35 with NaOH). Tissues are then dispersed in a series of incubations at 37°C in HEPES-buffered saline solution containing 1.2 mg/ml pancreatin and 0.14 mg/ml collagenase (Worthington). After centrifugation cells are re-suspended in Dulbecco's modified Eagle's medium/F-12 (Amersham Biosciences) containing 5% heat-inactivated horse serum, 0.1 mm ascorbate, insulin-transferring-sodium selenite media supplement, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.1 mm bromodeoxyuridine. The dissociated cells are pre-plated at 37 °C for 1 h and then diluted to 1 × 106 cells/ml and plated in culture dishes according to the specific experimental requirements.

siRNA transfection (non-virus)

Cardiac cells are grown in culture media described in our publications. SiRNAs COX-2 will be chemically synthesized using the T7 RNA polymerase-mediated transcription method (Silencer siRNA Construction kit; Ambion, Austin, TX http://www.ambion.com/catalog/ProdGrp.html?fkApp=25&fkProdGrp=248). The rat-specific siRNA COX-2 target sequence is 5'-aaggttcttctgaggagagag-3' (GenBank: NM_017232**:** position in gene sequence: 494: Antisense siRNA: 5'-AAGGTTCTTCTGAGGAGAGAGCCTGTCTC -3'. Sense siRNA: 5'-AACTCTCTCCTCAGAAGAACCCCTGTCTC -3'). A nonspecific control siRNA will be used as a negative control. COX-2 siRNA will be introduced into the cells using Lipofectamine TM RNAiMAX (Invitrogen) according to the manufacturer's instructions. After the transfection, the cells will be collected for the further experiments.

Constructions of Adenoviruses Harboring target iRNA and adenovirus Infection:

All empty adenovirus plasmids/vectors will be purchased from individual companies (see previously submitted documents). We will buy the adenovirus containing the individual target if the source is available. Viruses will be amplified in HEK293 cells. Here is one example of our constructions of adenovirus harboring target iRNA is CK2α iRNA:

The rat CK2α RNAi target sequence is 5′ -ATCAAGATGACTACCAGCT-3′ . Either a scrambled RNAi or empty vector will be used as a control (5′ -ATCAAGACTACGACTTCAG-3′). The specificity of the oligonucleotides was confirmed by comparison with all other sequences in GenBank™ using nucleotide BLAST. There was no homology to other known rat DNA sequences. The adenoviruses harboring these RNAi were constructed using the pSilencer ™ adeno 1.0-CMV System (Ambion http://www.ambion.com/jp/techlib/tn/105/7.html) according to the kit's instructions. The effects of the construct on CK2α expression will be tested. Reference showed that it could significantly inhibit the expression of CK2α in rat.

Cardiomyocytes will be infected with adenoviruses as described elsewhere with our modification. Following the infection, cells will be treated with various agents for further experiments.

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	Dr. Morris Karmazyn
DEPARTMENT	Physiology & Pharmacology
ADDRESS	Dept of Physiology & Pharmacology
PHONE NUMBER	519-661-3872
EMERGENCY PHONE NUMBER(S)	519-471-4330
EMAIL	Morris.Karmazyn@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Medical Sciences Bldg.__ Room(s)_242,247,259

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

FUNDING AGENCY/AGENCIES: CIHR and HSFO_____
GRANT TITLE(S):__; Role of Leptin in Cardiac Hypertrophy and Heart Failure CIHR)_____
Role of Adenosine in Myocardial Hypertrophy and Heart Failure (HSFO)__
Nitric Oxide as an Endogenous Antihypertrophic Factor (HSFO)__

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

Name	UWO E-mail Address	Date of Biosafety Training
Venkatesh Rajapurohitam	Venkatesh.rajaprohitam@schulich.uwo.ca	June 2009
Tracey Gan	tgan@uwo.ca	June 2009
Jim Haist	James.Haist@schulich.iwo.ca	June 2009
Melissa Moey	mmoey@uw.ca	Oct 2009
Eduoardo Martinez	Eduardo.martinez@schulich.uwo.ca	May 2009
Cathy Huang	Cathy Xiauling.Huang@schulich.uwo.ca	June 2009
Nazo Said	nsaidfa@uwo.ca	July 2008
Seiichi Taniai	New employee – does not yet have email	Not yet completed

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

The laboratory produces and uses purified heart cells from 1 to 5 day-old Sprague Dawley rats. Other cell types isolated during the process are killed by autoclaving soon after the initial production. The cells are kept in an incubator or a laminar flow hood for 4 to 6 days. One endpoint of cell use is to kill the cells and scrape out of the dishes as much as possible for measurement of cellular DNA or protein. The other endpoints are photography of the dishes or measurement of light or fluorescence in dishes. After wards all dishes are autoclaved. Pipets, pipet tips, filters, and other equipment used in the preparation and use of the cells are also autoclaved.

Please include a one page research summary or teaching protocol.

The majority of experiments will be done on either in cultured neonatal rat ventricular myocytes particularly to examine hypertrophic responses and in rats subjected to coronary artery ligation. Our laboratory has experience with most of the methodology proposed; otherwise key collaborations have been established to assure successful completion of the proposed studies. It is anticipated that this study will provide a comprehensive picture of the role of leptin in heart failure and provide a detailed and comprehensive evaluation of underlying mechanisms. The following studies will be performed to study leptin: 1. We will determine the effect of leptin inhibition on extracellular remodelling in the postinfarcted rat myocardium and determine the role of gender in these responses. 2. We will determine the role of mitochondria and the regulation of mitochondrial function in the postinfarcted myocardium and the influence of leptin inhibition. 3. As cell to cell communication represents a key defect in heart failure, we will determine the relationship between inhibition of both hypertrophy, remodelling/heart failure, and myocardial connexin expression. 4. We will study the mechanisms underlying the salutary effect early brief administration of a leptin receptor antagonist against the development of myocardial remodelling and heart failure. To determine the role of NO as an endogenous antihypertrophic factor and to study the underlying mechanisms involved, the experiments proposed here will be carried out using animals subjected to coronary artery ligation in which the heart failure process can be monitored. In addition, to obtain a better understanding of mechanisms underlying hypertrophic responses we will use cultured adult rat ventricular myocytes which will be studied after in vivo treatment or exposed to hypertrophic stimuli in culture and in which specific mechanisms can be closely studied. Among these mechanisms include the role of the RhoA/ROCK system as a target for the effects of NO To study the effect of obesity, we will produce obesity in rats by feeding them a high fat diet for either a 12 or 24 week period. We will also carry out experiments in which mice with specific genetic nitric oxide synthase (NOS)isoform deletions will be subjected to coronary artery ligation and resultant heart failure to determine the contributions of specific NOS isoforms to the remodelling process. Thus, overall, we will utilize pharmacological, physiological, cellular and molecular approaches to determine the role of the NO system in the hypertrophic and heart failure process. This study, we believe, will provide a comprehensive assessment into the role of NO in the hypertrophic program and delineate the mechanistic bases for these complex effects. The following represent the major general research goals aimed at determining the role of adenosine in cardiac hypertrophy and heart failure: 1. To determine the potential modulatory role of adenosine receptor agonists on other (non PE) G-protein linked hypertrophic factors 2. To assess the cellular mechanisms underlying the antihypertrophic effect of adenosine receptor activation in cultured myocytes with particular emphasis on the role of the RhoA/ROCK system. 3. To determine whether adenosine receptor expression is altered in hypertrophied cultured myocytes and postinfarcted remodelled myocardium. 4. To determine the response of adenosine receptor activation to hypertrophy in the postinfarcted myocardium 5. To determine the antihypertrophic effects of adenosine receptor modulation in the aging myocardium and to determine whether aging influences adenosine receptor expression profiles and the response to insult. 6. To determine whether treatment delay with adenosine receptor modulators can reverse the maladaptive response and affect the remodelling and heart failure process.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES xOx NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	xOx Yes O No	Rat heart	Aus protocol 2004-020 BS
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	xOx Yes O No	cardiomyocytes	Prepared in our lab
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 2+ O 3

JS

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES xOx NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used____Sprague Dawley rat_____

6.3 AUS protocol # _____2009-020_____

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

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

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

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

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

8.5 How much of the toxin is stored*? _____

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

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

9.0 Insects

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

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

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

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

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

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

11.0 Import Requirements

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

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

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

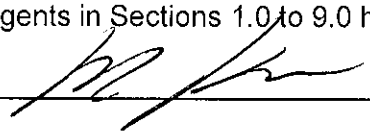
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

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

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

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

SIGNATURE 

13.0 Containment Levels

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

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

O YES, permit # if on-campus_____
O NO, please certify
xOx NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: November 30, 2010

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

_____
_____
_____

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

See attached e-mail -JS
_____

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 13 Dec 2010

2) Safety Officer for the University of Western Ontario
SIGNATURE: JStanley
Date: Dec 23/10

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

Approval Number: BIO-UWO-0171 Expiry Date (3 years from Approval): December 22, 2013

Special Conditions of Approval:

-------- Original Message --------
Subject: Fwd: biohazards 14.3 (BARF for Karmazyn lab)
Date: Thu, 09 Dec 2010 10:38:46 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: James Haist <james.haist@schulich.uwo.ca>, Morris Karmazyn <morris.karmazyn@schulich.uwo.ca>

Thanks Jim

Also, for Section 2, I assume that the rat hearts/cardiomyocytes are obtained from work done under AUS protocol 2009-020?
Question 2.4 would then be Level 1.

Regards,
Jennifer

-------- Original Message --------
Subject: biohazards 14.3
Date: Thu, 02 Dec 2010 10:09:40 -0500
From: James Haist <James.Haist@schulich.uwo.ca>
To: jstanle2@uwo.ca

Hello Jennifer,
re section 14.3 , please fill the form with the following

"If there is a small accidental exposure such as a needlestick injury, a small amount of bleeding is encouraged when there is a cut or scrape, followed by thorough washing with hand soap and warm water, and a bandage (a small first aid kit is kept in the laboratory) is applied if appropriate. If an injury is any more serious, we can proceed to take a person to staff health in the University Community Centre or to emergency at University Hospital both of which are a five minute walk away."

This seems to be the information requested, but if there is anything else, please let me know.
Thanks.

Jim

Jim Haist
Dep't of Physiology & Pharmacology
U.W.O.
phone 661-2111-ext 86699
e-mail James.Haist@schulich.uwo.ca