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

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

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

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

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

PRINCIPAL INVESTIGATOR	Dr. Lisa M. Hoffman
DEPARTMENT	Imaging
ADDRESS	268 Grosvenor St.
PHONE NUMBER	X61472
EMERGENCY PHONE NUMBER(S)	(519) 434-5009
EMAIL	lhoffman@lawsonimaging.ca

Location of experimental work to be carried out: Building(s) LHRI, SJHC Room(s) F4-127a; B5-251; F5-104; C0-232

*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:The Stem Cell Network, CIHR, NSERC, and a Lawson Internal Research Fund (IRF)

GRANT TITLE(S): SCN: Non-Invasive Assessment of Stem Cell Therapy in Dystrophic Mice; CIHR: Application of Angiogenic Growth Factors to Enhance Satellite Cell Therapy for Duchenne Muscular Dystrophy; NSERC: Development of Non-Invasive Cellular Tracking To Assess Muscle Homeostasis; IRF: Stem Cell Therapy for DMD-Associated Cardiomyopathy

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

Name	UWO E-mail Address	Date of Biosafety Training
Astrid Chamson-Reig	achamson@lawsoimaging.ca	21-Nov-08
Kelly Gutpell	kgutpell@uwo.ca	16-Sep-09
Hamed Moazami	hamed.moazami@alumni.utoronto.ca	
Jennifer Hadway	jhadway@awsonimaging.ca	13-Jun-05
		* Added by OHS

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.

2.0 Cell Culture
2.2 Primary cells:

Human: MDA-MB-435. From the Koropatnick lab, derived from human breast carcinoma, obtained from Dr. Donna Goldhawk at Lawson. This line is to be used to demonstrate proof-of-principle that MagA-expressing cells (line engineered by D. Goldhawk) migrate in vitro in a specified magnetic field.

Rodent: Muscle satellite cells (SCs) harvested from our transgenic murine line housed at LHRI, not a biohazard.

2

Established cells:

Rodent:

(1) C2C12 cells - ATCC # CRL-1722, mouse muscle myoblast, ATCC biosafety level 1. This line is radiolabelled with a PET substrate, then injected intramuscularly into the hindlimbs and imaged on a uPET scanner. Following scans, muscle is removed for ex vivo analyses/histology, and the carcass disposed of in biohazardous waste.

(3) H9-C2 – ATCC # CRL-1446, rat cardiomyoblast line, obtained from Dr. Frank Prato's group at Lawson, ATCC biosafety level 1. This line is radiolabelled with a PET substrate, then injected intramuscularly into the heart and imaged on a uPET scanner. Following scans,muscle is removed for ex vivo analyses/histology, and the carcass disposed of in biohazardous waste.

Please include a one page research summary or teaching protocol.

See next page ...

The purpose of this project is to develop (1) stem cell therapies for the
treatment of Duchenne muscular dystrophy (DMD), a severe muscle degenerative disease, and (2) imaging techniques for the non-invasive targeting of either transplanted skeletal muscle stem cells /myocardia function post-implant. Cells for transplant are harvested from transgenic
mouse lines engineered to express reporter genes for detection on appropriate scanners. Recipient mice are murine models of Duchenne muscular dystrophy (mdx mice lack functional dystrophin; mdx:utrn-/- mice
lack both functional dystrophin and are null for utrophin). Computed tomography (CT), is comparable to an x-ray with images being reconstructed
on a computer. MicroPET (uPET) uses a radioactive substance that is injected into a vein, then taken up by the muscle and imaged on the uPET
scanner. High-frequency ultrasound (HFU) uses sound waves to image the muscles. Each of these technologies allow us to assess changes in the same
animal over time. Attempts to improve cell therapy for treatment include
the use of growth factors that for new blood vessels.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? XO YES O 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

Cardiotoxin (see attached sheets)

Do you use microorganisms that require a permit from the CFIA? XO YES O NO
If YES, please give the name of the species. Lentivirus (use described in separate BHARF)
What is the origin of the microorganism(s)? ___InVitrogen_____
Please describe the risk (if any) of escape and how this will be mitigated: _Described in separate BHARF_____
_____
_____
Please attach the CFIA permit.
Please describe any CFIA permit conditions:
_____
_____
_____

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.coli chemically-competent cells	O Yes XO No	O Yes XO No	O Yes XO No	Used to amplify DNA. Not for use directly in cells or in animals	InVitrogen	XO 1 O 2 O 2+ O 3
Stb13 and Top 10			es o			O 1 O 2 O 2+ O 3
			es o			O 1 O 2 O 2+ O 3
	O No	O No	es 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? XO 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	XO Yes O No	MDA-MB-435 (breast cancer line, obtained from D. Goldhawk, UWO)	Not applicable

Rodent	XO Yes O No	1. Muscle Satellite Cells (SCs)(murine, obtained from myogenin-tk transgenic mice 2. cardiac side population (CSP) cells (muring, obtained from aMHC-tk transgenic mice	2008-067
Non-human primate	O Yes XO No		
Other (specify)	O Yes XO No		

MDA-MB-235 cell line

2.3 Please indicate the type of established ce

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	O Yes O No			
Rodent	XO Yes O No	C2C12 H9-C2	Level 1 Level 1	ATCC ATCC
Non-human primate	O Yes XO No			
Other (specify)	O Yes XO 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 XO 1 O 2 O 2+ O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		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 O NO If no, please proceed to Section 5.0

Yes

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation/transfection
1. Stbl3 chemically-competent E.Coli	*ViraPower Lentiviral Expression Vector*	*InVitrogen*	*Fluc-mrfp-sr39tk*	*expression of reporter genes*
2. TOP10 chemically-competent E.coli	*pBS; fluc-mrfp-sr39tk-reporter viral construct*	*obtained from QP Feng, UWO; Sanjiv Gambhir, Stanford*	*Fluc-mrfp-sr39tk*	*expression of reporter genes*

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Lentivirus	*See 4.2*	*See 4.2*	*See 4.2*	*See 4.2*

* Please attach a Material Safety Data Sheet or equivalent.
NB: this vector has ONLY been used to generate the myogenic-tk murine line. NO viral support vectors were used, making this DNA construct NO DIFFERENT than a standard plasmid with a bacterial backbone. All work that involves the INFECTION of primary and/or established cell lines for implantation into animals is detailed in a SEPARATE BHARF.

4.4 Will genetic sequences from the following be involved? As outlined above, the below aspects of th backbone were NOT employed, although the vector does contain the selected elements.

- HIV XO YES, please specify (see attached sheets)_____O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____X O NO
- SV 40 Large T antigen XO YES O NO
- E1A oncogene XO YES (HEK cells) 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? XO YES O NO

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

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

NO HEK 293 cells per PI May 25, 2011

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES XO 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
Please attach a full description of the biological agen

No oncogenes - per PI May 25, 2011

5.2 Will the biological agent be able to replicate in the

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? XO YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used__Mice_____

6.3 AUS protocol # __2008-067_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: __XO NO, NOT on this BHARF

6.5 Will the agent(s) be shed by the animal: O YES XO 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 XO No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES XO No

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

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

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

_____

8.0 Biological Toxins

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

If YES, please name the toxin(s)_Cardiotoxin (CTX) (see attached sheets)

8.2 _____

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_(mouse i.v.) 1.5 mg/kg_____

8.4 How much of the toxin is handled at one time*? 50 ul of 10uM stock_____

8.5 How much of the toxin is stored*? __1 mg_____

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

CTX is well-reported for use to locally damage muscle, aiding in engraftment of transplanted cells

*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 XO 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 XO 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? XO YES, please give country of origin_____ O NO
If no, please proceed to Section 12.0

NB: again, use of lentivirus to infect cells for transplantation is detailed in a separate BHARF. The SOLE use of the lentiviral backbone was to construct an expression vector that contains reporter genes for non-invasive imaging in mice. There is absolutely NO ability of the vector alone to infect cells, etc.

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

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

11.4 Has the import permit been sent to OHS? XO YES, please provide permit # _P-13043; A-2007-00178-4_____ 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. XO 1 O 2 O 2+ O 3

NB: while the lentiviral work (separate BHARF) is a Level 2+ BioSafety Level, the work being conducted with cells and agents listed on the current BHARF are exclusively Level 1.

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

XO YES, date of most recent biosafety inspection: _____
O NO, please certify
● NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

14.0 Procedures to be Followed

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

Lab coats, safety goggles and gloves will be standard dress when handling CTX. While all primary cells, cell lines and bacterial strains (for amplifying DNA) outlined in this BHARF are Level 1, personnel will don lab coats and gloves during handling.

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

Should accidental splashes, needlesticks, etc. occur, the designated eyewash and first aid stations will be utilized, followed by a visit to OHS, if necessary.

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

SIGNATURE _____ Date:May 6, 2011

15.0 Approvals

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

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: May 9/2011

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

Special Conditions of Approval:

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

E. coli

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Info on Cell Line(s)

Cell Biology

ATCC® Number:	CRL-1772™	Order this Item	Price:	$279.00

Designations:	C2C12
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	myoblast 
Source:	**Tissue:** muscle **Strain:** C3H **Cell Type:** myoblast;
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.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Comments:	This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903] The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953] Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427] Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

Cell Biology

ATCC® Number:	**HTB-129™**	Order this Item	Price:	**$279.00**

Designations:	MDA-MB-435S
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	spindle shaped 
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion
Cellular Products:	tubulin; actin
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.
Isolation:	**Isolation date:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

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

Cell Biology

ATCC® Number:	**CRL-1446™** Order this Item	Price:	**$279.00**

Designations:	H9c2(2-1)
Depositors:	W Carlisle
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	myoblast
Source:	**Strain:** BD1X **Organ:** heart **Tissue:** myocardium
Cellular Products:	myokinase; creatine phosphokinase; myosin
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.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Receptors:	acetylcholine, expressed
Age:	embryo
Comments:	H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue by B. Kimes and B. Brandt and exhibits many of the properties of skeletal muscle. Myoblastic cells in this line will fuse to form multinucleated myotubes and respond to acetylcholine stimulation. Fusion occurs faster if the serum concentration in the medium is reduced to one percent.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Related Links

NCBI Entrez Search
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Login Required

Product Information Sheet

– MSDS –

MATERIAL SAFETY
DATA SHEET

Note: this product is distributed in so minor quantities that, despite a possibly high level of toxicity, its potential hazard on the environment is negligible.

Cat. No. L81 02 December 6, 1994 Revised J.T. St. Joseph's Aug 31, 2009

CARDIOTOXIN

SECTION I IDENTIFICATION

Chemical Name: CARDIOTOXIN
Synonym: CYTOTOXIN
Formula: 60-amino acid peptide with 4 S-S bridges.

SECTION II HAZARDOUS INGREDIENTS DATA

Hazardous Components: Same as section I (single compound)

SECTION III PHYSICAL DATA

Boiling Point : N/A		Volatile by Volume : N/A	
Vapor Pressure at Temperature : N/A		Evaporation Rate (Butyl Acetate = 1) : N/A	
Vapor Density : N/A		Specific Gravity : N/E	
Solubility in water : Good		Melting Point : N/A	
Appearance and odor : Crystalline or amorphous powder.			

SECTION IV EXPLOSION AND FIRE HAZARD DATA

Flash Point: : N/A Flammable Limit: N/A
Test Mode : —

Extinguishing Media: Water, carbon dioxide, dry chemical powder, foam.
Special Fire Fighting Procedure: Firefighters must wear self-contained breathing apparatus and fully protective equipment.

Unusual Fire and Explosion Hazards: Smoke or fumes from burning may be toxic or irritating.

N/A = Not Applicable N/E = Not Established

SECTION V HEALTH HAZARD

Biological activity: Protein Kinase C inhibitor.

Toxicity: LD_{50} (mice, i.v.): 1.5 mg/kg
Route(s) of Entry: Inhalation: N/E Skin: N/A Ingestion: Yes
Health Hazards (acute and chronic): Toxic.
Medical Conditions Generally Aggraved by exposure: N/E

Signs and Symptoms of Exposure: N/E.

Emergency and First Aid Procedures:
Skin contact : Wash affected area with copious amounts of water.
Eye Contact : Flush eyes with water for at least 15 minutes.
Inhalation : Remove to fresh air. Give oxygen or artificial respiration as needed.
Ingestion : Call physician immediately.

Seek medical treatment if discomfort persists.

SECTION VI REACTIVITY DATA

Stability : Stable.
Conditions to Avoid : N/E
Incompatibility : N/E
Hazardous Decomposition Products : N/E
Hazardous polymerisation : N/A

SECTION VII SPILL OR LEAK PROCEDURES

Steps to be Taken in case Material is Released or Spilled: Wear self-contained breathi apparatus, rubber boots and gloves. Sweep up. Do not raise dust. Wash and ventilate spill after pickup is complete. Do not allow material or wash water to enter natural waterway.

Waste Disposal Method: Mix waste with a combustible carrier and burn in a suitable equi chemical incinerator.

Disposal must comply with Federal, state and local regulations.

SECTION VIII SPECIAL PROTECTION INFORMATION

Respirator Protection : Mechanical filter type.
Ventilation : Mechanical.
Protective Gloves : Nitrile rubber (J.T. St. Joseph's Aug 31, 2009)
Eye Protection : Goggles.
Other Protective Equipment : Store dry in tight container.
Should be handled only by qualified, experienced professionals.

SECTION IX SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storage: Keep storage container tightly closed prolonged or frequent exposure. Wash thoroughly after handling.

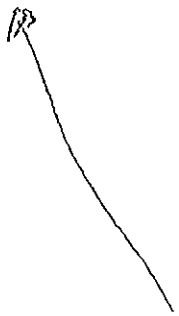
Toxin Info

Cardiotoxin will be used to damage the muscle to improve SC transplantation. This treatment will by itself increase the success of myoblast tramsplantation. If used in combination with irradiation for the initial transplantation, this will further improve the transplantation success. Cardiotoxin injection will be repeated 2-3 weeks after cell implantation, will further increase the success of transplantation, and will induce a proliferation of the SCs initially transplanted. We do not expect pain to be caused by the injection, there should just be localized irritation and minor muscle damage enough to help the cell transplant. However the mouse will be checked daily to be sure that it is not having any walking problems or unexpected outcomes from the injection.

----- Original Message -----
From: "Jennifer Stanley" <jstanle2@uwo.ca>
To: "Jennifer Hadway" <jhadway@lawsonimaging.ca>
Cc: "Lisa Hoffman" <lhoffman@lawsonimaging.ca>
Sent: Monday, September 14, 2009 5:05 PM
Subject: Re: Biohazard Modification Form: Lee (AUS 2008-067)]

Hi Jen
Can you tell me
1. LD50 for the toxin (and species) Mice 1.5mg/kg iv
2. How much of the toxin is handled at one time? 50ul of 10uM stock
3. How much of the toxin is stored? 1mg

Thanks
Jennifer



1.5 mg/kg × 50 kg / 1 person = 75 mg.

d.
Conservative
(overall person)

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cardiotoxin
Proposed Use Dose:	50 µg *
Proposed Storage Dose:	1000 µg
LD_{50} (species):	1500 µg

Calculation:

1500 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 75000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 7500

Comments/Recommendations:

*** Usage Dose given as volume. Calculations assume the same density as water.**