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	Douglas William Hamilton
DEPARTMENT	Oral Biology/Dentistry
ADDRESS	Dental Sciences Building Rm 0065
PHONE NUMBER	81594
EMERGENCY PHONE NUMBER(S)	519-601-3209; 519-777-3661
EMAIL	dhamil2@uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 0064

*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, NSERC, WIF, CFI
GRANT TITLE(S): Centre for the Study of Biomaterials and Tissue regeneration (CFI); Role of the Matricellular Protein Periostin in Cutaneous Wound Healing (CIHR); Biology of Cell-Surface Interactions (NSERC); Role of the Matricellular Protein in the Repair of human Chronic Skin Wounds (WIF).

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

Name	UWO E-mail Address	Date of Biosafety Training
Weiyan Wen	wwen@uwo.ca	18-Jan-2008
Christopher Elliott	cellio43@uwo.ca	11-Oct-2008
Paul Prowse	pprowse@uwo.ca	16-Oct-2009
Shawna Kim	Shawna.kim87@gmail.com	06-Oct-2008
Eric Bellis	ebellis@uwo.ca	15 Jun 2010
Kang Hyun Keem	kkeem@uwo.ca	Nov 2010

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.

E-coli chemically compentent cells: This work is performed in DSB 0064. They are transformed using heat shock and expanded in the presence of ampiciilin. Glycerol stock solutions are stored at -80C. The remainder of the cells are lysed for plasmid preps. The bacteria are destroyed using bleach and autoclaving. All samples are stored at -80 in a locked room. Waste is disposed of in accordance with University guidelines.

Plasmids: NIH 3T3 cells are transiently transfected with plasmids expressing wither b-galactosidase, luciferase and ampicillin. Transfected cells are not stored. Termination of experiments involves cell lysis in Trizol, formaldehyde fixation or protein extraction in RIPA buffer. All samples are stored at -80C in a locked room. All material in contact with cells is autoclaved and glassware washed in bleach.

Cells derived from humans: All cells are used for basic research purposes. We analyze the influence of biomaterials on gene and protein expression. Cells are cultured in a class II hood and maintained in incubators in the PIs laboratory. Cells are kept frozen in liquid nitrogen in a locked dewar and in a locked room. Cells that are to be disposed are placed in cavicide prior to autoclaving. All cell culture materials are autoclaved and disposed of according to University guidelines.

Biological toxins: All biological toxins are stored in Room 0064 in a -20 freezer. Toxins are used at the specified dilution to inhibit cell-signaling pathways. At the termination of experiments, media containing any remnants of the toxins is siphoned off into cavicide prior to disposal.

Please include a one page research summary or teaching protocol.

Project 1: Hip and knee replacement with metal implants is a commonly used treatment for patients with arthritis. However, the lifespan of these devices are often limited by osteoclasts which resorb the bone at the interface between the bone and the implant, loosening the implant. Revision surgery is then required. Alterations in the topography of the implant surface have been commonly utilized to encourage bone formation, but how such features influences osteoclasts is not known. We will investigate using cultured cells and animal models, topographic modifications of implant surfaces that could initially enhance bone formation, while preventing bone resorption at later times.

Project 2: Non-healing dermal wounds and fibrotic scars are a significant cause for morbidity and the identification of new therapeutic targets to help regenerate skin or prevent fibrosis would be of great clinical significance. Periostin, a matricellular protein that is expressed in several collagen-rich tissues, has been suggested to mediate differentiation of fibroblasts to myofibroblasts and regulate collagen fibrillogenesis, processes essential for normal cutaneous repair. These findings support our contention that **periostin is an important mediator of skin repair and remodeling** and could represent a therapeutic target to enhance repair dermal wounds or prevent fibrotic scars forming. However, the biological functions of periostin in healthy and injured skin are as yet unknown. To determine the function and regulation of periostin in skin, we will use periostin knockout and wild type mice *in vivo*, and periostin knockout and wild type fibroblasts *in vitro.* We will then assess the pathways upstream of periostin expression.

Project 3: Musculoskeletal and connective-tissue disease and injury are among the biggest causes of chronic disability in the Canadian community and are major factors impacting on health as people age. For example, approximately 1 million Canadians suffer from osteoporosis, costing Health Canada an estimated $1.3 billion annually. However, periodontal disease remains the most common connective-tissue disorder in Canada, with approximately 60% of the population affected. The limitations of current (2nd generation) biomaterials, has led to the exponential expansion of the field of tissue engineering. Although the term was first coined in the late eighties, tissue engineering is still in its infancy, with considerable gaps in our knowledge persisting. For example, bone and cartilage produced in the laboratory do not have sufficient strength to function in the body, and are not clinically viable. The project leader, Dr. Hamilton, proposes to address these challenges by establishing an innovative research program to investigate different factors governing regeneration of musculoskeletal and connective tissues. The requested infrastructure will enable Dr Hamilton to address why tissues formed in vitro exhibit poor structure and function, and thereby develop cell-scaffold composites appropriate for clinical use. He will employ a comprehensive, step-wise approach, utilizing state-of-the-art cell and molecular biology techniques, imaging, unique material fabrication processes, application of biomechanics, as well as genetically modified mouse models, to dissect out how different cell populations and proteins contribute to regeneration of musculoskeletal tissues.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES ☒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
E.Coli DH5α competent cells	O Yes ☒No	O Yes ☒No	O Yes ☒No	0.5	Invitrogen	☒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? O YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒Yes O No	Skin from human limbs and foreskin; gingival from human	Not applicable
Rodent	O Yes O No	Skin - mice Gingiva - mice ears	
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 X No		
Rodent	X Yes O No	NIH 3T3	Sigma
Non-human primate	O Yes X No		
Other (specify)	O Yes X 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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/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)	London Health Sciences Victoria Hospital (fresh)	O Yes X Unknown		O 1 X O 2+ O 3
Human Organs or Tissues (preserved)	Oral pathology Pathology Dept.	Not Applicable		Not Applicable

preserved specimen → fixed

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? X YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X 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
E. Coli DH5α	pGL3-Basic	Promega	Luciferase Reporter Gene	Ampicillin (Ampicillin resistance expression of luciferase)

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

4.5 Will virus be replication defective? O YES O NO N/A

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____ N/A

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mus Musculus, Rattus norvegicus

6.3 AUS protocol # 2008 097

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

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

_____

_____

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 O 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? X YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) Phalloidin, Wortmannin, Actinomycin D, Cycloheximide
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 Ph → 2mg/kg Cyclo → 2mg/kg Wortmannin → 16 mg/kg Actino → 7.2 mg/kg

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

8.5 How much of the toxin is stored*? 1mg Phall, 1mg Wortmannin, 500g Cycloheximide (0064), 500g Cycloheximide (0064), Actinomycin D → 2mg.

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ X 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 O 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:

_____

_____

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

_____

_____

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? YES 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? YES 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? YES, please give country of origin_____ NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ 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 D. W Hamilton

13.0 Containment Levels

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

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

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

14.0 Procedures to be Followed

See Next Page (Section 14)

14.1 Please describe additional risk reduction measures wil[illegible] measures, that are unique to this agent.

_____
_____
_____

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:

_____
_____

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 D.W Hamilton Date: 30/11/2010

15.0 Approvals

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

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

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

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

Special Conditions of Approval:

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 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
O 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:_____

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.

All fridges/freezers are in a locked room. Samples/forms only accessable to workers in Hamilton lab.

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

Person will have wound cleaned, it will be reported to Health & Safety. Hospital treatment if required.

15.0 Approvals

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

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

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

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

Special Conditions of Approval:

Subject: EXPIRED Biological Agents Registry Form (Hamilton)
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Thu, 09 Dec 2010 10:02:41 -0500
To: Douglas Hamilton <Douglas.Hamilton@schulich.uwo.ca>

Thanks Doug

The form was on my desk when I got in this morning.

I have a couple of questions:

1. How much of the toxins (wortmannin, actinomycin D and cycloheximide) do you use/handle at a time?
2. Question 14.2 was not answered (please address by e-mail):

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:

3. There is a signature missing (Section 12.0).

Regards,
Jennifer

-------- Original Message --------
Subject:Re: Fwd: Biological Agents Registry Form (Hamilton)
Date:Mon, 17 Jan 2011 17:35:27 -0500
From:Douglas Hamilton <Douglas.Hamilton@schulich.uwo.ca>
To:Jennifer Stanley <jstanle2@uwo.ca>

Jennifer,

Perhaps a fillable PDF form would be better than having to rewrite the form, it makes corrections much easier.

Table 3.2 - please clarify whether the tissues are fixed or fresh frozen. - There is no section in the table to indicate this specifically. It already says which ones are unpreserved Vs preserved which is all the form asks.

It is recommended that cycloheximide be stored in two separate locations because the storage amount is more than one lethal dose - Done
Please clarify the change results from the transfection of the gene (Table 4.2). Ampicillin resistance.
The E.coli in Table 1.2 is Level 1 only. - Done

regards
Doug.

New Info

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 11/25/2010
Date Updated: 02/04/2006
Version 1.2

Section 1 - Product and Company Information

Product Name	NIH 3T3 CELLS, MOUSE SWISS NIH EMBRYO FIBROBLAST
Product Number	93061524
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
EUROPEAN COLLECTION OF CELL CULTURES, NON-HUMAN / NON-PRIMATE	None	No

Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
DIMETHYL SULFOXIDE	67-68-5	10	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Readily absorbed through skin. Target organ(s): Eyes. Skin.

HMIS RATING

HEALTH: 0*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING

HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

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): Combustible liquid. Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL
Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Cover with dry-lime, sand, or soda ash. Place in covered containers using non-sparking tools and transport outdoors. Ventilate area and wash spill site after material pickup is complete.

ENVIRONMENTAL PRECAUTION(S)
Avoid contaminating sewers and waterways with this material.

Section 7 - Handling and Storage

HANDLING
User Exposure: Do not breathe vapor. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

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.
Skin-Specific: Chemical resistant apron.

GENERAL HYGIENE MEASURES
Wash contaminated clothing before reuse. 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 EXOTHERMIC REACTIONS

Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

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. Readily absorbed through skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Eyes. Skin.

Section 12 - Ecological Information

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. This combustible material may be burned 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

US Statements: Readily absorbed through skin. Target organ(s): Eyes. Skin.

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 or packing slip for additional terms and conditions of sale.

pgl3-basic vector

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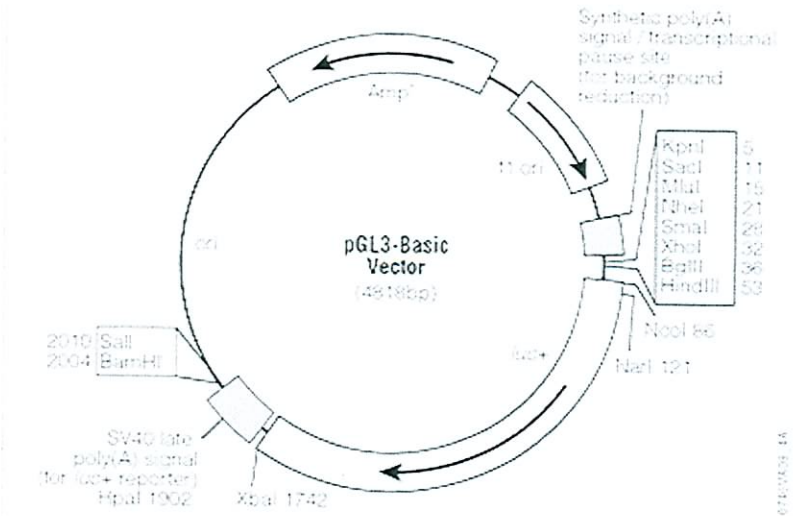

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Phalloidin
Proposed Use Dose:	0.002 µg
Proposed Storage Dose:	9 µg
LD_{50} (species):	2000 µg

Calculation:

2000 µg/kg x 70 kg/person

Dose per person based on LD_{50} in µg = 140000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 14000

Comments/Recommendations: OK

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Wortmannin
Proposed Use Dose:	µg
Proposed Storage Dose:	1000 µg
LD $_{50}$ (species):	18000 µg

Calculation:

18000 µg/kg x 70 kg/person

Dose per person based on LD $_{50}$ in µg = 1260000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 126000

Comments/Recommendations: OK

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Actinomycin D
Proposed Use Dose:	µg
Proposed Storage Dose:	2000 µg
LD $_{50}$ (species):	7200 µg

Calculation:

7200 µg/kg x 70 kg/person

Dose per person based on LD $_{50}$ in µg = 504000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 50400

Comments/Recommendations:

OK

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cycloheximide
Proposed Use Dose:	µg
Proposed Storage Dose:	1000000 µg
LD $_{50}$ (species):	2000 µg

Calculation:

2000 µg/kg x 70 kg/person

Dose per person based on LD $_{50}$ in µg = 140000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = ***14000***

Comments/Recommendations:
storage dose exceeds calculated LD50