

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
 Approved Biohazards Subcommittee: October 14, 2011
 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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	Dr. Sean Gill
DEPARTMENT:	Medicine
ADDRESS:	800 Commissioners Rd. E, VRL A6-134, N6A 5W9
PHONE NUMBER:	519-685-8500 ext 55443
EMERGENCY PHONE NUMBER(S):	519-854-6977
EMAIL:	sgill8@uwo.ca

Location of experimental work to be carried out :

Building : Victoria Research Labs (VRL)	Room(s): 6th FL Lab & A6-118
Building : _____	Room(s): _____
Building : _____	Room(s): _____

***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: **Parker B. Francis Foundation and Ontario Thoracic Society**

GRANT TITLE(S): **The Protective Role of TIMP3 in Acute Lung Injury**
TIMP3 Regulates Macrophage Function following Acute Lung Injury

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Ryan Bird	rbird3@uwo.ca	Jul 7, 2012/Lawson
Matt Tsang	mtsang24@uwo.ca	TBD
Lefeng Wang	N/A	Jul 9, 2012/Lawson
Marta Rohan	N/A	Apr 28, 2011/Lawson
_____	_____	_____

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

Research in my laboratory focuses on the resolution of inflammation and fibrosis following lung injury. Acute lung injury (ALI) is characterized by profound inflammation and tissue injury often resulting from trauma or severe pulmonary infection. Although ALI survival rates have improved in recent years, 25 to 40% of cases remain fatal, and of those patients that do survive, persistent inflammation and fibrosis can result in continued pulmonary complications. I have determined that tissue inhibitor of metalloproteinases 3 (TIMP3) serves to restrict ongoing neutrophil influx during the repair process, and is thereby integral to the resolution of inflammation. Additionally, I have demonstrated that TIMP3 moderates the fibrotic response following lung injury, and that in the absence of TIMP3, fibrosis is enhanced.

To establish the mechanisms through which TIMP3 mediates resolution of inflammation and fibrosis following lung injury, my group utilizes both in vivo and in vitro techniques. My lab uses multiple models of direct lung injury, including bleomycin (a model often used to study chronic lung injury and fibrosis). These models are performed on wild type (or control mice) and mice lacking TIMP3 (Timp3^{-/-} mice). Techniques such as flow cytometry and immunohistochemistry are then used to examine how specific cell populations are affected by the presence or absence of TIMP3 during lung injury. We also use tissue culture to elucidate the mechanism through which TIMP3 regulates the function of specific cell populations (i.e. BMDMs). Current work is focused on understanding the role that TIMP3 has in controlling macrophage polarization and apoptosis following ALI.

1.0 Microorganisms

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

Full Scientific 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
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Mouse Bone Marrow	Mehta 2011-026
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	CCL-1 (L-929)	BSL1	ATCC
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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 1 2 2+ 3

Additional Comments: _____

3.0 Use of Human Source Materials

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

3.2 Indicate 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		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

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

*** Please attach a plasmid map.*

****No Material Safety Data Sheet is required for the following strains of E. coli:*

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

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.*

4.3.1 Will virus be replication defective? YES NO

4.3.2 Will virus be infectious to humans or animals? YES NO

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? YES, number: NO PENDING

7.0 Animal Experiments

7.1 Will live animals be used? YES NO

Is it okay to use another PI's AUS protocol #?

7.2 Name of animal species to be used **Mice**

7.3 AUS protocol # **Mehta 2011-026**

7.4 List the location(s) for the animal experimentation and housing. **Victoria Research Labs Animal Facility**

7.5 Will any of the agents listed in section 4.0 be used in live animals
 NO YES, specify:

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES NO - If NO, please proceed to section 9.0

8.2 Will live animals be used? YES NO

8.3 If YES, please specify the animal(s) used:

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

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Lipopolysaccharide (LPS) & Bleomycin Sulfate**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD₅₀ (specify species) of the toxin or hormone **LPS: 7670µg/kg IV (mouse); Bleomycin Sulfate: 210mg/kg IV (Mouse) & 2000mg/kg Oral (Mouse)**

9.4 How much of the toxin or hormone is handled at one time*? **LPS: 10mg (only when aliquoting); Bleomycin Sulfate: 30mg (only when aliquoting)**

9.5 How much of the toxin or hormone is stored*? **LPS: 10mg; Bleomycin Sulfate: 30mg**

9.6 Will any biological toxins or hormones be used in live animals? YES NO
If **YES**, Please provide details:

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: **We use LPS isolated from E. coli to stimulate our BMDMs. LPS is purchased in 10mg quantities, reconstituted at 2mg/mL in saline and aliquoted into 500µL aliquots.**

10.0 Insects

10.1 Do you use insects? 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? Initiate and maintain colony, give location:

"One-time" use, give location:

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

10.7 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:

11.0 Plants

- 11.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 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.

An X in the check box indicates you agree with the above statement...
Enter Your Name Sean Gill **Date:** June 29, 2012

14.0 Containment Levels

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

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

- YES, location and date of most recent biosafety inspection:
- NO, please certify
- NOT REQUIRED for Level 1 containment

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

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? YES NO

If YES please describe:

15.2 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:

The researcher will be removed from the area and the exposed tissue will immediately be washed with copious amounts of water for at least 15 minutes. Any contaminated clothing/shoes will be removed. After rinsing the exposed area, the researcher will contact the Occupational Health nurse at Victoria Hospital (ext. 52286). The contaminated area will be disinfected with bleach.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html>

An X in the check box indicates you agree with the above statement...

Enter Your Name Sean Gill *Date:* June 29, 2012

15.4 Additional Comments: _____

16.0 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: _____ *July 31, 2012*

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

Special Conditions of Approval:

Bleomycin:

The paragraph specific to Bleomycin is lacking a description of the personal protective equipment or work practices around the use of this substance. Section 97 of O.Reg.67/93 (Health Care and Residential Facilities) requires that there be written measures and procedure to work with antineoplastic drugs.

If Lawson has such a document then I would assume that the researchers would work within that program to ensure worker safety and that the program is sound.

If no document exists, the following would be best practices for working with Bleomycin.

- a. Preparation of the solution, administration and any other potential aerosolizing activities should be conducted in a BSC. If this is not practicable then a N95 disposable respirator should be worn. A surgical mask will not protect against aerosolization of the solution.
 - b. Double nitrile gloves are highly recommended to avoid skin contact. If possible, the gloves should be longer than wrist-length or the outer glove taped to the sleeve of the lab coat.
 - c. Safety goggles should also be worn, if not used in BSC
 - d. If needles will be used to instill the bolus of Bleomycin, I'd suggest using engineered safety needles.
 - e. Bedding needs to be disposed of as biohazardous waste for incineration.
 - f. Work areas must be cleaned thoroughly between tasks. Equipment or contaminated materials that cannot be cleaned must be disposed of as hazardous waste.
2. Question 14.2 indicates that the lab has been certified by OHS but the "location and date of the most recent biosafety inspection" was not inserted.
3. Note that employees handling frozen vials of the cell lines packed in liquid nitrogen should wear either a full-face respirator or a N99 disposable respirator with a face shield, if not handled in BSC.

Cell Line Designation:
NCTC clone 929 (L cell; L-929)
ATCC® Catalog No. CCL-1™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Mus musculus* (mouse)

Strain: C3H/An

Tissue: subcutaneous connective tissue; areolar and adipose

Age: 100 days

Gender: male

Morphology: fibroblast

Growth properties: adherent

VirusSuscept: vesicular stomatitis (Indiana); encephalomyocarditis

VirusResist: poliovirus 1, 2, 3; coxsackievirus B5; polyomavirus

Tumorigenic: yes, in immunosuppressed mice

AntigenExp: H-2k

Depositors: W.R. Earle

Comments: NCTC clone 929 (Connective tissue, mouse) Clone of strain L was derived in March, 1948. Strain L was one of the first cell strains to be established in continuous culture, and clone 929 was the first cloned strain developed. The parent L strain was derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse. Clone 929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain.

Tested and found negative for ectromelia virus (mousepox).

Karyotype: modal chromosome number = 66; range = 65 to 68. There were approximately 20 to 30 marker chromosomes present in each metaphase spread. A high percentage of those markers were common to most analyzed cells. A long metacentric chromosome with secondary constriction was noted in 77/100 cells.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bml4/bml4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). *It is important to avoid excessive alkalinity of the medium during recovery*

of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

5. Add appropriate aliquots of cell suspension to new culture vessels.

Subcultivation ratio: 1:2 to 1:8

6. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- horse serum to a final concentration of 10%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

ATCC tested horse serum is available as ATCC Catalog No. 30-2040 (500ml) and ATCC Catalog No. 30-2041 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Fisher EM et al. **Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome.** Cell 63: 1205-1218, 1990 PubMed: 91084849

Sanford KK et al. **The growth in vitro of single isolated tissue cells.** J. Natl. Cancer Inst. 9: 229-246, 1948

Sugarman BJ et al. **Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro.** Science 230: 943-945, 1985 PubMed: 86044518

Standard practice for direct contact cell culture evaluation of materials for medical devices. ASTM Standard Test Method F813-83 (reapproved 1988)

Standard test method for agar diffusion cell culture screening for cytotoxicity. ASTM Standard Test Method F895-84 (reapproved 1990)

Biological reactivity tests, in-vitro. U.S. Pharmacopeia, 23rd rev., <87>, pp. 1697-1699, 1995
J. Natl. Cancer Inst. 14: 655, 1953

Earle WR et al. **Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells.** J. Natl. Cancer Inst. 4: 165-212, 1943

J. Natl. Cancer Inst. 12: 133, 1951

Cancer Res. 16: 162, 1956

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SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 10.07.2012
Date Updated: 13.02.2012
Version 1.7

Section 1 - Product and Company Information

Product Name LIPOPOLYSACCHARIDES FROM ESCHERICHIA
COLI 0111:B4, PURIFIED BY
GEL-FILTRATION CHROMATOGRAPHY
Product Number L3012
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
LIPOPOLYSACCHARIDES FROM ESCHERICHIA COLI 0111:B4, PREPARED BY GEL-FILTRATION CHROMATOGRAPHY	None	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Harmful.
Pyrogen. May cause fever. Do not use if skin is cut or scratched.
Wash thoroughly after handling.

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 breathing becomes difficult,
call a physician.

DERMAL EXPOSURE

In case of skin contact, flush with copious amounts of water for
at least 15 minutes. Remove contaminated clothing and shoes.
Call a physician.

EYE EXPOSURE

In case of contact with eyes, flush with copious amounts of
water for at least 15 minutes. Assure adequate flushing by
separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT

N/A

AUTOIGNITION TEMP

N/A

FLAMMABILITY

N/A

EXTINGUISHING MEDIA

Suitable: 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.

Section 6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

STORAGE

Store at 2-8°C

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

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 dust mask type N95 (US) or type P1 (EN 143) respirator.

Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

Section 9 - Physical/Chemical Properties

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.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Multiple Routes: May be harmful by inhalation, ingestion, or skin absorption.

CONDITIONS AGGRAVATED BY EXPOSURE

The toxicological properties have not been thoroughly investigated.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT

Proper Shipping Name: None

Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION

Symbol of Danger: Xn
Indication of Danger: Harmful.

US CLASSIFICATION AND LABEL TEXT

Indication of Danger: Harmful.
US Statements: Pyrogen. May cause fever. Do not use if skin is cut or scratched. Wash thoroughly after handling.

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.
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Material Safety Data Sheet

Version 4.1
 Revision Date 10/26/2010
 Print Date 07/10/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : **Bleomycin sulfate, from *Streptomyces verticillus***

Product Number : B5507

Brand : Sigma

Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
 2149 Winston Park Drive
 OAKVILLE ON L6H 6J8
 CANADA

Manufacturer : Sigma-Aldrich Corporation
 3050 Spruce St.
 St. Louis, Missouri 63103
 USA

Telephone : +1 9058299500

Fax : +1 9058299292

Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300

Preparation Information : Sigma-Aldrich Corporation
 Product Safety - Americas Region
 1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Lungs, Reproductive system.

WHMIS Classification

D2A Very Toxic Material Causing Other Toxic Reproductive hazard
 D2B Effects Mutagen

GHS Classification

Carcinogenicity (Category 2)

GHS Label elements, including precautionary statements

Pictogram



Signal word Warning

Hazard statement(s)
 H351 Suspected of causing cancer.

Precautionary statement(s)
 P281 Use personal protective equipment as required.

HMIS Classification

Health hazard: 0
 Chronic Health Hazard: *
 Flammability: 0
 Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.

Ingestion

May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Blenoxane
Bleo
Blexane

CAS-No.	EC-No.	Index-No.	Concentration
Bleomycin, sulfate (salt)			
9041-93-4	232-925-2	-	-

4. FIRST AID MEASURES

General advice

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact

Wash off with soap and plenty of water. Consult a physician.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability

Not flammable or combustible.

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Explosion data - sensitivity to mechanical impact

no data available

Explosion data - sensitivity to static discharge

no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions

Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up

Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling

Avoid contact with skin and eyes. Avoid formation of dust and aerosols.

Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

strongly hygroscopic

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection

Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls

Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	crystalline
Colour	no data available

Safety data

pH	no data available
Melting/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available

Water solubility	ca.20 g/l
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

no data available

Conditions to avoid

no data available

Materials to avoid

Strong oxidizing agents

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50

Inhalation LC50

no data available

Dermal LD50

no data available

Other information on acute toxicity

LD50 Intraperitoneal - rat - 240 mg/kg

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

Laboratory experiments have shown mutagenic effects.

Carcinogenicity

This product is or contains a component that has been reported to be possibly carcinogenic based on its IARC, ACGIH, NTP, or EPA classification.

Limited evidence of carcinogenicity in animal studies

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a

carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

Overexposure may cause reproductive disorder(s) based on tests with laboratory animals.

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)

no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)

no data available

Aspiration hazard

no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure

Anorexia., Nausea, Vomiting, Fever, Headache, Lowered blood pressure, Alopecia., Confusion.

Synergistic effects

no data available

Additional Information

RTECS: EC5991990

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability

no data available

Bioaccumulative potential

no data available

Mobility in soil

no data available

PBT and vPvB assessment

no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product

Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION**DOT (US)**

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

15. REGULATORY INFORMATION**DSL Status**

All components of this product are on the Canadian DSL list.

WHMIS Classification

D2A	Very Toxic Material Causing Other Toxic	Reproductive hazard
D2B	Effects	Mutagen

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION**Further information**

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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 Co., 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.



Western
UNIVERSITY · CANADA

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Lipopolysaccharide (LPS)
Proposed Use Dose:	10000 µg
Proposed Storage Dose:	10000 µg
LD₅₀ (species):	7670 µg

Calculation:
$7670 \mu\text{g/kg} \quad \times \quad 50 \text{ kg/person}$
Dose per person based on LD ₅₀ in µg = 383500
LD₅₀ per person with safety factor of 10 based on LD₅₀ in µg = 38350

Comments/Recommendations:



Western
UNIVERSITY · CANADA

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Bleomycin Sulfate
Proposed Use Dose:	30000 µg
Proposed Storage Dose:	30000 µg
LD₅₀ (species):	210000 µg

Calculation:	
210000 µg/kg	x 50 kg/person
Dose per person based on LD ₅₀ in µg = 10500000	
LD₅₀ per person with safety factor of 10 based on LD₅₀ in µg =	1050000

Comments/Recommendations: