

**THE UNIVERSITY OF WESTERN ONTARIO  
 BIOLOGICAL AGENTS REGISTRY FORM**  
**Approved Biohazards Subcommittee: October 14, 2010**  
**Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://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, 1<sup>st</sup> 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](mailto: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/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR James Lewis, Ruud Veldhuizen, and Cory Yamashita  
 DEPARTMENT Medicine  
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 EMAIL [jflewis@uwo.ca](mailto:jflewis@uwo.ca), [rveldhui@uwo.ca](mailto:rveldhui@uwo.ca), [cyamash@uwo.ca](mailto:cyamash@uwo.ca)

Location of experimental work to be carried out: Building(s) LHRI\_ Room(s)\_F4-117 for LPS, F4-124 for cell culture

\*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  
 GRANT TITLE(S): The Role of Pulmonary Surfactant in Acute Lung Injury and Progression to Multi-Organ Failure

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Lynda McCaig</u>	<u><a href="mailto:lmccaig@uwo.ca">lmccaig@uwo.ca</a></u>	<u>2004</u>
<u>Lijuan Yao</u>	<u><a href="mailto:jyao@uwo.ca">jyao@uwo.ca</a></u>	<u>2004</u>

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**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.**

LPS (lipopolysaccharide):

LPS powder is stored at 4 C fridge, after dissolve in saline, make aliquots and stored in -20C

We handle LPS powder in the fume hood with gloves, mask, lab coat and safety glasses on. When we use this saline dissolved LPS in animals we also wear gloves, lab coat and safety glasses.

The animals that have been used with LPS are disposed of in a biohazard waste box for incineration within the ACF.

Cell line:

MLE-12 (Mouse lung epithelial cells)

Cells are aliquoted and stored in the liquid nitrogen tank for storage

We perform all cell experiment in the cell culture room within F4-124 (Dr. David Hill's laboratory)

All wastes are collected in a biohazard box for incineration

**Please include a one page research summary or teaching protocol.**

The overall objective of research in our lab is to understand the mechanisms leading to Acute Lung Injury and to develop logical treatment strategy to improve the outcome of patients afflicted with this syndrome.

ALI is defined by the inability of the lung facilitating the diffusion of oxygen from the atmosphere into the blood. This type of lung dysfunction can occur in people of all ages due to one or more insult to the lung. Some of the more common initiating event leading to ALI are systemic infection, pneumonia, aspiration of gastric content and trauma. The reported incidence of ALI ranges from 15 to 79 cases per 100,000 person-years depending on the specific population studied, and has a mortality of approximately 40%. In addition, patient surviving ALI, have often required a prolonged stay in the Intensive Care Unit. As such, ALI represents a significant burden on our healthcare system.

From a research perspective, one of the important aspects of this disease that has limited our ability to develop appropriate therapies is the complexity of the disease. The multiple events involved in the development of the disease have been shown to contribute to a very complex, multi-factorial process which may vary among individual patients. To address this issue our lab has focused on specific common pathological processes such as the contribution of mechanical ventilation and the role of pulmonary surfactant.

Experimentally, our lab tries to use several complementary approaches to study the ALI from a physiological to a molecular level. At the physiological level we utilize animal models of ALI that reflect the human disease. Specifically we study the physiological effects of a small amount of acid instilled into the lung of mice and rats as models of gastric acid aspiration that occurs in humans. We also study the effect of mechanical ventilation, the main supportive therapy for ALI, on the lungs of mice and rats. Material from the animal experiments is used for advanced biophysical analysis such as atomic force microscopy and captive bubble surfactometry, as well as inflammatory measurements such as the determination of cytokines, adhesion molecules and NFkB activation. To

study more specific cellular aspects of lung injury we utilize various cell lines ( MLE12 and MLEC) to determine the effects of inflammation and cell stretch on cellular responses.

Lipopolysaccharide(LPS) is used in the experiments as a lung insult to make lung injury at a very low dose (1mg/kg). LPS is dissolved in saline and instilled into animal lungs. At the end of the experiment, animal bodies are sent to a biohazard waste box for incineration.

**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:

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="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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?

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 type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

may not otherwise be distributed, copied or disclosed. If you have received this e-mail in error, please notify the sender immediately via a return e-mail and destroy original message. Thank you for your cooperation.

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="radio"/> Yes <input type="radio"/> No			
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Mouse MLE-12	2	ATCC
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No			

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

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

### 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="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid	Lung lavage from Proteinosis Patients	<input type="radio"/> Yes <input checked="" type="radio"/> Unknown		1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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?  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 Transfected	Describe the change that results from transformation or transfection

\* 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?

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? No

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

4.5 Will virus be replication defective?  YES  NO

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

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

**5.0 Human Gene Therapy Trials**

5.1 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 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?  YES  NO

5.3 How will the biological agent be administered? \_\_\_\_\_

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

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used mice\_

6.3 AUS protocol # 2010-272\_

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

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify:  
\_LPS is instilled into the lungs of mice. 4 hours later mice are euthanized, this is to short a time for LPS to enter the systemic circulation and be excreted in urine or feces.

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 X No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

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

- ◆ Pound source dogs O YES X NO
◆ Pound source cats O YES X NO
◆ Cattle, sheep or goats O YES, please specify species \_\_\_\_\_ X NO
◆ Non-human primates O YES, please specify species \_\_\_\_\_ X NO
◆ Wild caught animals O YES, please specify species & colony # \_\_\_\_\_ X NO
◆ Birds O YES, please specify species \_\_\_\_\_ X NO
◆ Others (wild or domestic) X YES, please specify \_mouse\_\_\_\_\_ O 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? X YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) LPS

Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

What is the LD50 (specify species) of the toxin 7670 ug/kg IV in mice

8.4 How much of the toxin is handled at one time\*? \_We purchase 500mg at a time and we aliquot 2 mg which is then suspended in saline and aliquoted into 100uL aliquots. 100uL aliquots are frozen so that personnel can use in animal work.

8.5 How much of the toxin is stored\*? \_500 mg

8.6 Will any biological toxins be used in live animals? X YES, Please provide details: \_50 uL (containing 30ug LPS) is instilled intratracheally in mice. Mice are euthanized 4 hours later. O NO

\*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES X 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?  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  "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

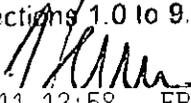
11.4 Has the import permit been sent to OHS?  YES, please provide permit # \_\_\_\_\_  NO

**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 

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 OMS for this level of containment?

X YES, date of most recent biosafety inspection: 2011/02/02  
O NO, please certify  
O 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.

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

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

Personnel will go immediately to Occupational Health at SJHC

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: Feb 9/11

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: 2011/02/02

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

E-mail

**Subject:** It's done  
**From:** Lijuan Yao <jyao@uwo.ca>  
**Date:** Mon, 14 Feb 2011 12:13:03 -0500  
**To:** jstanle2@uwo.ca

Hi Jennifer,

Thank you for the additional information.  
In answer to you questions,

1. We have added Lynda. She will only be involved with LPS work.
2. We put the MLE-12 cells in the table under 2.3, we couldn't find Table 3.1....
3. We have changed this to Level 2.
4. Opps. Thanks we now have Jim Lewis' signature here.
5. We have attached the MSDS for the LPS we use and for the MLE-12 cells (as required under table 2.3)

Thanks again for your help,  
Li Juan Yao



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## Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's [Material Transfer Agreement](#) or, in certain cases, an MTA specific institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

## Cell Biology

**ATCC® Number:** CRL-2110™ **Order this Item** **Price:** \$379.00

**Designations:** MLE 12

**Depositors:** JA Whitsett

**Biosafety Level:** 2 [CELLS CONTAIN PAPOVAVIRUS ]

**Shipped:** frozen

**Medium & Serum:** [See Propagation](#)

**Growth Properties:** adherent

**Organism:** Mus musculus, transgenic (mouse, transgenic)

**Morphology:** epithelial

**Source:** Organ: lung  
Strain: FVB/N  
Cell Type: epithelialSV40 transformed

**Cellular Products:** lung surfactant proteins B and C (SP-B, SP-C)

**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: 1992

**Tumorigenic:** Yes

**Age:** 5 month old

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TO- UWO-HR-Occ. Health P011/016

## Related Links

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[Related Cell Culture](#)

**Gender:** female

**Comments:** This line was established in 1992 by Kathryn A. Wikenheiser from pulmonary tumors in a mouse transgenic for the SV40 large T antigen under the control of the promoter region of the human surfactant protein C gene.  
The cells express the mRNA for large T antigen.  
Lung surfactant proteins B and C were detected.  
The cells secrete phospholipids in response to phorbol esters and ATP but not in response to forskolin.

**Propagation:** **ATCC complete growth medium:** HITES medium supplemented with 2% fetal bovine serum

HITES medium with 2% fetal bovine serum is formulated at the ATCC as follows:

- Dulbecco's medium : Ham's F12, 50:50 mix (ATCC 30-2006)
- Insulin 0.005 mg/ml
- Transferrin 0.01 mg/ml
- Sodium selenite 30 nM
- Hydrocortisone 10 nM
- beta-estradiol 10 nM
- HEPES 10 mM
- L-glutamine 2 mM (in addition to that in the base medium)
- fetal bovine serum 2%

**Atmosphere:** air, 95%; carbon dioxide (CO<sub>2</sub>), 5%

**Subculturing:** **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that 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 the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:5 to 1:10 is recommended

**Medium Renewal:** Twice per week

**Preservation:** **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO  
**Storage temperature:** liquid nitrogen vapor phase

**Doubling Time:** 7 to 9 hrs

**Related Products:** Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2006

**References:** 23101: Wikenheiser KA, et al. Simian virus 40 large T antigen directed by transcriptional elements of the human surfactant protein C gene produces pulmonary adenocarcinomas in transgenic mice. Cancer Res. 52: 5342-5352, 1992. PubMed: 1394139  
23539: Wikenheiser KA, et al. Production of immortalized distal respiratory epithelial cell lines from surfactant protein C/simian virus 40 large tumor antigen transgenic mice. Proc. Natl. Acad. Sci. USA 90: 11029-11033,

**SIGMA-ALDRICH****Material Safety Data Sheet**

Version 3.0  
 Revision Date 01/03/2009  
 Print Date 05/31/2010

**1. PRODUCT AND COMPANY IDENTIFICATION**

Product name : **Lipopolysaccharides, from *Escherichia coli* 026:B6**

Product Number : **L3755**  
 Brand : **Sigma**

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

Telephone : **+19058299500**  
 Fax : **+19058299292**  
 Emergency Phone # : **800-424-9300**

**2. COMPOSITION/INFORMATION ON INGREDIENTS**

CAS-No.	EC-No.	Index-No.	Concentration
<b>Lipopolysaccharides from <i>Escherichia coli</i> 026:B6</b>			
-	-	-	-

**3. HAZARD IDENTIFICATION****Emergency Overview**

**Other hazards which do not result in classification**  
**Pyrogen. May cause fever.**

**WHMIS Classification**

Not WHMIS controlled.

Not WHMIS controlled.

**HMIS Classification**

**Health Hazard:** 0  
**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.

**4. FIRST AID MEASURES**

**If inhaled**  
**If breathed in, move person into fresh air. If not breathing give artificial respiration**

**In case of skin contact**

Wash off with soap and plenty of water.

**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.

**5. FIRE-FIGHTING MEASURES****Flammable properties**

Flash point no data available

Ignition temperature no data available

**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.

**6. ACCIDENTAL RELEASE MEASURES****Personal precautions**

Avoid dust formation.

**Environmental precautions**

Do not let product enter drains.

**Methods for cleaning up**

Sweep up and shovel. Keep in suitable, closed containers for disposal.

**7. HANDLING AND STORAGE****Handling**

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

**Storage**

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

Recommended storage temperature: 2 - 8 °C

**8. EXPOSURE CONTROLS/PERSONAL PROTECTION**

Contains no substances with occupational exposure limit values.

**Personal protective equipment****Respiratory protection**

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**Hand protection**

For prolonged or repeated contact use protective gloves.

**Eye protection**

Safety glasses

**Hygiene measures**

General industrial hygiene practice.

0015/0016

**9. PHYSICAL AND CHEMICAL PROPERTIES**

**Appearance**

Form powder, lyophilized

**Safety data**

pH no data available

Melting point no data available

Boiling point no data available

Flash point no data available

Ignition temperature no data available

Lower explosion limit no data available

Upper explosion limit no data available

Water solubility no data available

**10. STABILITY AND REACTIVITY**

**Storage stability**

Stable under recommended storage conditions.

**Hazardous decomposition products**

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

**11. TOXICOLOGICAL INFORMATION**

**Acute toxicity**

no data available

**Irritation and corrosion**

no data available

**Sensitisation**

no data available

**Chronic exposure**

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.

**Signs and Symptoms of Exposure**

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

**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.

**12. ECOLOGICAL INFORMATION**

**Elimination information (persistence and degradability)**

no data available

**Ecotoxicity effects**

no data available

**Further information on ecology**

no data available

**13. DISPOSAL CONSIDERATIONS**

**Product**

Observe all federal, state, and local environmental regulations.

**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**

This product contains the following components that are not on the Canadian DSL nor NDSL lists.  
CAS-No.

Lipopolysaccharides from Escherichia coli 026:B6

**WHMIS classification**

Not WHMIS controlled.

Not WHMIS controlled.

**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.



**TOXIN USE RISK ASSESSMENT**

Name of Toxin:	LPS
Proposed Use Dose:	2000 µg ***
Proposed Storage Dose:	50000 µg
LD <sub>50</sub> (species):	7670 µg

<b>Calculation:</b>	
7670 µg/kg	x 50 kg/person
Dose per person based on LD <sub>50</sub> in µg = 383500	
<b>LD<sub>50</sub> per person with safety factor of 10 based on LD<sub>50</sub> in µg = 38350</b>	

**Comments/Recommendations:**

Too much stored; thus store in 2 places?

\*\*\*then suspended in saline and aliquoted into 100 uL aliquots; so the use dose is much less than 2000 ug