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:	**Wei-Yang Lu**
DEPARTMENT:	**Physiology and Pharmacology**
ADDRESS:	**Robarts Research Institute**
PHONE NUMBER:	**(519)663-5777 Ext. 24282**
EMERGENCY PHONE NUMBER(S):	**(519)663-5777 Ext. 24282**
EMAIL:	**wlu53@uwo.ca**

Location of experimental work to be carried out :

Building : **Robarts Research Institute** Room(s): **7255A1, 7234, 7250,**

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: **Canadian Institutes of Health Research; Canadian Cystic Fibrosis Foundation**

GRANT TITLE(S): **GABAergic regulations of airway epithelium in asthma; Understand the role of chloride channel GABA-A-receptor in CF lung disease and its relationship to CFTR protein**

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
Jason Wong	**jwong446@uwo.ca**	**Nov. 02, 2011**
Yan Luo	**yluo93@uwo.ca**	**Feb. 19, 2012**
Yun-Yan Xiang	**yxiang8@uwo.ca**	**Oct. 19, 2009**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

The cells will be handled in the biological safety cabinet at room 7255A-1 in combination with personal protective equipment (gloves, laboratory coats and safety glasses). All waste materials will be autoclaved in the Robarts Research Institute before disposal.

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

ϒ -Aminobutyric acid (GABA), a major neurotransmitter in the central nervous system (CNS), generates fast inhibition in central neurons via activation of GABA type A (GABAA) receptors. GABAA receptors are pentameric hetero-oligomers assembled from different subunits with 19 members [α(1–6), β(1–3), γ(1–3), δ, ε, θ, π and ρ (1-3)]. GABAA receptors are also widely expressed in non-neuronal cells in organs outside the CNS such as pancreas and ovaries. Recent studies, including ours, have demonstrated that GABAA receptors as well as the key enzyme for GABA synthesis, glutamic acid decarboxylase (GAD), are expressed in alveolar type II epithelial (ATII) cells isolated from rats and mice. Studies also show that GABAA receptors are expressed in immune cells including T-lymphocytes, macrophages and microglia. More recently, our laboratory found that GABAA receptor subunit proteins were expressed in neutrophils. My laboratory is studying the role of GABAA receptors in regulating the functions of theses non-neuronal cells.

Different GABAA receptors have unique physiological and pharmacological properties due to the distinct subunit compositions. For example, GABAA receptors that contain α5 subunit are sensitive to low concentration of GABA and mediate tonic inhibitory activities in central neurons. Interestingly we found that in mice lacking GABAA receptor α5 subunit exhibit altered innate immunity. To understand the cellular mechanisms of GABA regulation of innate immunity, we plan to carry out RT-PCR analysis of α5 subunit in different lines of non-neuronal cells, including A549, BV-2 and HL-60, thus determine which type(s) of non-neuronal cells express α5 subunit.

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
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 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	☐ Yes ☒ No		Not applicable
Rodent	☒ Yes ☐ No	**Rat Brain**	**2010-033**
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ 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	☒ Yes ☐ No	**A549** **Beas-2B** **HepG2** **HL-60** **IB3-1** **Mz-ChA-1**	**1** **2** **1** **1** **2** **1**	**ATCC** **ATCC** **ATCC** **ATCC** **ATCC** **UTSMS**
Rodent	☒ Yes ☐ No	**BV-2** **Clone 9**	**2** **1**	**RRI** **ATCC**
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

**Please attach a Material Safe… …re information, see www.atcc.org)*

2.4 For above named cell ty… …uired ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Level 1 and 2

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		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction)		☐ Yes		☐1 ☐2

or other Body Fluid		☐ Unknown		☐ 2+ ☐ 3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐ 1 ☐ 2 ☐ 2+ ☐ 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 **v-raf, v-myc**
- 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 If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **C57BL/6,BALB/C,SpragueDawley/Wistar**

7.3 AUS protocol # **20120-033, 2010-038, 2010-285**

7.4 List the location(s) for the animal experimentation and housing. **HSACF, REB**

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: **None of the above cell lines will be used for animal experiments**

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) **gamma-Aminobutyric acid (GABA), Muscimol, Picrotoxin**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **GABA: 12,680mg/kg (mouse); Muscimol: 45mg/kg (rat); Picrotoxin: 15mg/kg (mouse)**

9.4 How much of the toxin or hormone is handled at one time*? **GABA: 3mg; Muscimol: 24ug; Picrotoxin: 2ug**

9.5 How much of the toxin or hormone is stored*? **GABA: 10g; Muscimol: 10mg; Picrotoxin: 5g**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **GABA: 150ug/g body weight; Muscimol: 1.2ug/g body weight; Picrotoxin: 0.1ug/g body weight**

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

Additional Comments: _____

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 Wei-Yang Lu ***Date:*** May 28, 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: **Rm 7255A-1, January 11, 2011**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-RRI-0056**

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:
In the event of a spill: If the spill is able to do it safely, the decontamination and clean-up procedures as recommended in "Emergency Procedures, Western University Biosafety Guidelines and Procedures Manual" will be followed (which is available in each room of my lab). If the spill is large or a nature that can not be handled by people in the lab, we will call 911 using lab phone.

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 Wei-Yang Lu ***Date:*** May 28, 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: Ronald Hoschouer
Date: May 30, 2012

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

Special Conditions of Approval:

Cell Line Designation: A549
ATCC Catalog No. CCL-185™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: lung carcinoma
Age: 58 years
Gender: male
Ethnicity: caucasian
Morphology: epithelial
Doubling time: about 22 hours
Growth Properties: adherent
DNA profile (STR analysis)
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
TH01: 8,9.3
TPOX: 8,11
vWA: 14
Products: keratin
Depositors: M. Lieber
Comments: This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway
The cells are positive for keratin by immunoperoxidase staining.
Karyology: This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%.
There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

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,* 4th 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; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.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 submersed 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 to7 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*

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

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% CO_2 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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 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).

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 .Cultures can be established at between 2 x 10(3) and 1x10(4) viable cells/cm2. Maintain cultures at a cell concentration between 6x10(3) and 6 x 10(4) cells/cm2; not exceeding 7x10(4) cells/cm2

Subculture Ratio: 1:3 to 1:8.

6. Incubate cultures 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

Fluid change cells two to three times per week.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

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 are available in the catalog at www.atcc.org)

Lieber M et al. **A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells.** Int. J. Cancer 17: 62-70, 1976 PubMed: 76120014

Giard DJ et al. **In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors.** J. Natl. Cancer Inst. 51: 1417-1423, 1973 PubMed: 74054240

Mayr GA and Freimuth P. **A single locus on human chromosome 21 directs the expression of a receptor for adenovirus type 2 in mouse A9 cells.** J. Virol. 71: 412-418, 1997 PubMed: 97138339

Goodrum FD and Ornelles DA. **The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle.** J. Virol. 71: 548-561, 1997 PubMed: 97138357

St. Geme JW et al. **Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils.** J. Bacteriol. 178: 6281-6287, 1996 PubMed: 97047989

Horikami SM et al. **The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication.** Virology 222: 383-390, 1996 PubMed: 96400146

Huang S et al. **Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells.** J. Virol. 70: 4502-4508, 1996 PubMed: 96256761

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Cell Line Designation: BEAS-2B
ATCC® Catalog No. CRL-9609™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: lung; bronchus; normal; epithelial; virus transformed
Morphology: epithelial
Growth properties: adherent
Depositors: The United States of America
Tumorigenicity: The cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.
Comments: Epithelial cells were isolated from normal human bronchial epithelium obtained from autopsy of a non-cancerous individual. The cells were infected with an adenovirus12-SV40 virus hybrid (Ad12SV40) and cloned. BEAS-2B cells retain the ability to undergo squamous differentiation in response to serum, and can be used to screen chemical and biological agents for ability to induce or affect differentiation and/or carcinogenesis. The cells stain positively for keratins and SV40 T antigen.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th 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; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

Cell lines and hybridomas deposited for patent purposes are not always screened for contamination, antibody production or characterized by the ATCC. Release of a culture, the use of which may be claimed in a patent, from the ATCC during the effective term of any such patent is not meant to carry with it, and does not grant any license, express or implied, under any patent, or the right to use a culture in any process described in a patent.

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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 submersed 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.*

Note: The culture flasks used should be **pre-coated** with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin dissolved in BEBM medium (see references : U.S. Pat. 4,885,238 and Lechner, J.F. and LaVeck, M.A. A serum-free method for culturing normal human bronchial epithelial cells at clonal density. J. Tissue Culture Methods 9: 43-48, 1985, below).

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 centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant.

4. Resuspend the cell pellet in the complete culture medium at the dilution ratio recommended in the specific batch information and dispense into a **pre-coated** T-25 cm^2 culture flask.

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 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.

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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% CO_2 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 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to **pre-coated** 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

These cells should be **subcultured before reaching confluence** since confluent cultures rapidly undergo squamous terminal differentiation.

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Add 2.0 to 3.0 ml of 0.25% Trypsin - 0.53mM EDTA solution containing 0.5% polyvinylpyrrolidone (PVP) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 10 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.

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

4. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.

5. Discard supernatant and resuspend cells in fresh growth medium. Inoculate new flasks at 1500 to 3000 cells per cm^2. The culture flasks used should be **pre-coated** with a mixture of 0.01mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01mg/ml bovine serum albumin dissolved in BEBM .

6. Place culture flasks 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

Every 2 to 3 days.

Flask Coating

1. Prepare a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin (BSA) dissolved in culture medium. Store pre-prepared Coating Solution at 4°C in cold room for up to 3 months.
2. For a growth area of 75 cm^2, add 4.5 ml of the fibronectin/collagen/BSA solution and rock gently to coat the entire surface.
3. Incubate the freshly coated vessel(s) in a 37°C incubator overnight (it is preferable to use tissue culture vessels with tightened, plug-seal caps to prevent evaporation during the coating process).
4. Store coated flasks with solution at room temperature, light protected, up to 1 month. Suction off solution before plating cells.

Complete Growth Medium

The base medium for this cell line (BEBM) along with all the additives can be obtained from Lonza/Clonetics Corporation as a kit: BEGM, Kit Catalog No. CC-3170. ATCC does not use the GA-1000 (gentamycin-amphotericin B mix) provided with the BEGM kit.

This medium is formulated for use with a 5% CO_2 in air atmosphere.

Cryoprotectant Medium

Complete growth medium plus 1% PVP and 7.5% 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

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Journal of Neuroimmunology, 27 (1990) 229–237
Elsevier

JNI 00935

Immortalization of murine microglial cells by a v-*raf*/v-*myc* carrying retrovirus

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(Received 12 September 1989)
(Revised, received 8 November 1989)
(Accepted 8 November 1989)

Key words: Microglia; Phagocytosis; Cell line; Oncogenes

Summary

A murine cell line (BV-2) has been generated by infecting primary microglial cell cultures with a v-*raf*/v-*myc* oncogene carrying retrovirus (J2). BV-2 cells expressed nonspecific esterase activity, phagocytic ability and lacked peroxidase activity. Such cells secreted lysozyme and, following appropriate stimulation, also interleukin 1 and tumor necrosis factor. Furthermore, BV-2 cells exhibited spontaneous anti-*Candida* activity and acquired tumoricidal activity upon treatment with interferon-γ. Phenotypically, BV-2 cells resulted positive for MAC1 and MAC2 antigens, and negative for MAC3, glial fibrillary acidic protein (GFAP) and galactocerebroside (GC) antigens. Since BV-2 cells retain most of the morphological, phenotypical and functional properties described for freshly isolated microglial cells, we can conclude that J2 virus infection has resulted in the immortalization of active microglial cells.

Introduction

It is well established that cells with phagocytic properties, the so-called 'ameboid' microglia, appear in the brain during the late stage of embryogenesis. However, by the late postnatal period such cells disappear, likely giving rise to the non-phagocytic 'ramified' microglia, whose role is still unknown (Del Rio Hortega, 1932; Ling, 1981; Murabe and Sano, 1982; Oehmichen, 1982). After brain injury or bacteria/virus infection(s) in adult rodents, ameboid cells with phagocytic ability can be detected again. Whether these cells are blood-recruited monocytes and/or 'reactivated' microglia is still controversial (Imamoto and Leblond, 1977; Oehmichen, 1982; Perry et al., 1987). Moreover, in humans, microglial cells are involved in pathological processes of the central nervous system (CNS), such as in demyelinating diseases and human immunodeficiency virus (HIV) infections (Esiri and McGree, 1986; Woodroofe et al., 1986; Price et al., 1988).

Many attempts have been made to provide in vitro models for further studies on microglia. Using newborn as well as adult brain tissues, primary

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Depositors:	ME Kaighn
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Rattus norvegicus* deposited as *Rattus* sp.
Morphology:	epithelial
Source:	**Organ:** liver **Strain:** Sprague-Dawley **Disease:** normal
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:** 1968
Age:	4 weeks
Gender:	male
Comments:	Clone 9 (K-9) is an epithelial cell line isolated in 1968 from normal liver taken from a young male rat. The line has been used for studies of in vitro carcinogenesis and is useful clonal assays for screening sera and other nutritional supplements.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

Subculturing:

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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 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

21872: . Gene expression and carcinogenesis in cultured liver. New York: Academic Press; 1975.
22425: Weinstein IB, et al. Growth and structural properties of epithelial cell cultures established from normal rat liver and chemically induced hepatomas. Cancer Res. 35: 253-263, 1975. PubMed: 162864

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Cell Line Designation: Hep G2
ATCC Catalog No. HB-8065™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: liver; hepatocellular carcinoma
Age: 15 years
Gender: male
Ethnicity: caucasian
Morphology: epithelial
Growth properties: adherent
Tumorigenic: the cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium.
Cellular Products: alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen;
complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein)
Receptors expressed: insulin; insulin-like growth factor II (IGF II)
Depositors: Wistar Institute
Comments: The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to gramoxone (oxidative stress). There is no evidence of a Hepatitis B virus genome in this cell line
Karyotype: Modal number = 55 (range = 50 to 60); has a rearranged chromosome 1.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA from this line is available as ATCC HB-8065D™ (10µg)

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,* 4th 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; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

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The above culture was deposited in the ATCC in connection with a **patent** application. Copies of U.S. Patents may be obtained from the Commissioner of Patents, U.S. Patent and Trademark Office, Box 9, Washington, D.C. 20231.
This material is cited in a U.S and/or other Patent and may not be used to infringe the patent claims.

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 submersed 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 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 culture flask. *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% CO_2 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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 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 the cell suspension to new culture vessels.
Subcultivation Ratio: 1:4 to 1:6.

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Fluid change twice 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:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 and ATCC Catalog No. 30-2021 (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.

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(additional references are available in the catalog at www.atcc.org)

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Product Information Sheet for ATCC® CCL-240™

Cell Line Designation: HL-60
ATCC Catalog No. CCL-240™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: peripheral blood; acute promyelocytic leukemia; promyeloblast
Age: 36 years
Gender: female
Ethnicity: Caucasian
Morphology: myeloblastic
Growth properties: suspension
Tumorigenic: yes, form colonies in semi-solid media and produce subcutaneous myeloid tumors in nude mice.
Oncogene: myc +
Reverse Transcriptase: negative
Receptors expressed: complement; Fc
Products: tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-alpha, TNF alpha), after stimulation with phorbol myristic acid
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 13,14
D13S317: 8,11
D16S539: 11
D5S818: 12
D7S820: 11,12
TH01: 7,8
TPOX: 8,11
vWA: 16

Depositors: R.C. Gallo
Comments: HL-60 is a promyelocytic cell line derived by S.J. Collins, et al. Peripheral blood leukocytes were obtained by leukopheresis from a 36-year-old Caucasian female with acute promyelocytic leukemia. HL-60 cells spontaneously differentiate and differentiation can be stimulated by butyrate, hypoxanthine, phorbol myristic acid (PMA, TPA), dimethylsulfoxide (DMSO, 1% to 1.5%), actinomycin D, and retinoic acid.

The cells exhibit phagocytic activity and responsiveness to chemotactic stimuli. The line is positive for myc oncogene expression.
Karyotype: The stemline chromosome number is pseudodiploid with the 2S component occurring at 6.2%. Five markers (M2 through M6) were common to most S metaphases. DM's, which varied in numbers per cell, occurred in all metaphases karyotyped.
HSR chromosomes were not detected
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA from this line is available as ATCC CCL-240D™ (10µg).
Total RNA from this line is available as ATCC CCL-240R™ (100 µg).

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,* 4th 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; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.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 submersed 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).

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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 to7 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% CO_2 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

2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 ml of this medium.

3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2-3 X 10^5 viable cells/ml in the shipping medium.

4. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Cultures can be maintained by addition of fresh medium. Alternatively, cultures can be subcultured by total medium replacement by centrifugation with subsequent resuspension at 1 X 10^5 viable cells/ml. Maintain cultures at a cell concentration between 1 X 10^5 and 1 X 10^6 cells/ml. Do not allow the cell concentration to exceed 1 X 10^6 cells/ml.

Medium Renewal

Add medium every 2 to 3 days depending on cell density.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 20%

This medium is formulated for use with a 5% CO_2 in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

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 at www.atcc.org)

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Cell Biology

ATCC® Number:	CRL-2777™ Order this Item	**Price:**	**$338.00**
Designations:	IB3-1 [JHU-52]		
Depositors:	PL Zeitlin		
Biosafety Level:	2 [Cells contain SV40 and Adenovirus 12 DNA viral sequences]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	**Organ:** bronchus **Disease:** cystic fibrosis **Cell Type:** epithelial immortalized with Ad12-SV40 hybridimmortalized with adenovirus 12 - SV40 virus hybrid (Ad12-SV40)		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Restrictions:	Part of the Johns Hopkins Special Collection		
Isolation:	**Isolation date:** 1992		
Cytogenetic Analysis:	modal chromosome number = 80 to 90. There are an average of four chromosome 7 per cell. The phenylalanine 508 deletion in the gene coding for the cystic fibrosis transmembrane regulator is present on at least one chromosome. [70685]		
Age:	7 years		
Gender:	male		
Ethnicity:	White		
Comments:	IB3-1 (ATCC CRL-2777) is an immortalized cell line created in 1992 from a primary culture of		

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bronchial epithelia cells isolated from a patient with cystic fibrosis. The culture was transformed with a hybrid virus, adeno-12-SV40 [PubMed: 1849726]. The IB3-1 are deficient in cyclic AMP-medicated protein kinase A activation of chloride conductance, which is diagnostic of Cystic Fibrosis [PubMed: 7679117]. Genotypically, the cell line is a compound heterozygote containing the delta F508 mutation and a nonsense mutation, W1282X, with a premature termination signal [PubMed: 10518596].The cells stain positively for SV40 T antigen [PubMed: 1849726].They can be used for studies of the mutant cystic fibrosis transmembrane regulatory protein and its interaction with the chloride channel.The S9 cell line (ATCC CRL-2778) and the C38 cell line (ATCC CRL-2779) were derived from the IB3-1 cell line.The CF phenotype present in the IB3-1 cells was corrected in the S9 and C38 cell line by transfection with wild-type adeno-associated viral cystic fibrosis transmembrane conductance regulator (AAVCFTR).

Propagation:

ATCC complete growth medium: LHC-8 Basal Medium (Invitrogen catalog #12679-015), 95%; fetal bovine serum, 5%
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The flasks used should be precoated with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin dissolved in culture medium.

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 precoated culture vessels. An inoculum of 3 X 10(3) to 8 X 10(3) viable cells/cm2 is recommended. Do not exceed 1 X 10(5) cells/cm2..
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 4 X 10(3) and 4 X 10(4) cells/cm2.
Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended
Medium Renewal: Two to three times weekly

Preservation:

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

Doubling Time:

29 hrs

Related Products:

source culture:ATCC JHU-52
derivative:ATCC CRL-2778
recommended serum:ATCC 30-2020
derivative:ATCC CRL-2779

References:

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Human biliary epithelial cell line Mz-ChA-1, as provided by the UT Southwestern Medical School, Children's Medical Center, Pediatric Gastroenterology and Hepatology, 5323 Harry Hines Boulevard, Dallas, TX, 25390-9063, USA.

L Murphy
Lise Murphy
Manager, Licensing Program
Pathogen Regulation Directorate

NOVEMBER 05, 2010
Date

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Journal of Hepatology, 1985; 1: 579–596
Elsevier

HEP 0063

Biliary Adenocarcinoma

Characterisation of Three New Human Tumor Cell Lines

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(Received 17 January, 1985)
(Accepted 11 April, 1985)

Summary

Three human cell lines from adenocarcinomas of the extrahepatic biliary tract were established in permanent tissue culture. Mz-ChA-1 and Mz-ChA-2 were cultured from mechanically dissociated gallbladder adenocarcinoma metastases and SK-ChA-1 was grown from malignant ascites of a patient with primary adenocarcinoma of the extrahepatic biliary tree. Cell doubling times in tissue culture are 3–4 days for Mz-ChA-1 and approximately 2 days for Mz-ChA-2 and SK-ChA-1. All three tumour cell lines were successfully transplanted to nude mice, inducing progressive tumour growth. Histologically, nude mouse tumours resembled the original adenocarcinomas. In vitro formation of gland-like structures were regularly seen in Mz-ChA-1 and Mz-ChA-2 but only occasionally in SK-ChA-1. All three cell lines formed contacts through interdigitating processes with desmosomes and junctional complexes. On scanning electron microscopy, an abundance of microvilli was seen at the cell surfaces. Chromosome analyses of all three tumour cell lines showed a wide range of numerical abnormalities and presence of marker chromosomes. Mz-ChA-1 appears to be highly differentiated with cells producing mucus. Mz-ChA-2 synthesizes components of complement C2, C3 and C5, while Mz-ChA-1 and SK-ChA-1 produce only C3 in detectable quantities. In addition, Mz-ChA-2

This work was supported by grants from the Deutsche Forschungsgemeinschaft Kn-180/3, Di-245/3 and Sonderforschungsbereich 107.

* To whom reprint requests should be sent: Dr. Alexander Knuth, I. Medizinische Klinik und Poliklinik, Johannes Gutenberg-Universität Mainz, Langenbeck Strasse 1, D-6500 Mainz, F.R.G., Tel. 06131/17-2275.

supernatants are positive for ferritin and α_1-fetoprotein, but not CEA; while Mz-ChA-1 and SK-ChA-1 produce only CEA. Supernatants of all three cell lines are positive for N-acetyl neuraminic acid (NANA), phosphohexoisomerase (PHI) and LDH, and negative for α_2-macroglobulin, α_1-anti-trypsin, γ-GT, AP, coeruloplasmin, haptoglobin and albumin. A high cloning efficiency renders these new tumour cell lines suitable for continued studies on clonal heterogeneity in malignant tumours. The establishment of these cell lines in tissue culture facilitates further studies on the biology of upper gastrointestinal tract cancer in man.

Introduction

Biliary adenocarcinoma is an uncommon cancer that is often discovered accidentally at the time of cholecystectomy for cholelithiasis. In women, about 67% are gallbladder carcinomas, while in men bile duct cancers account for 60% [1].

The aetiology and pathogenesis are poorly understood, but gallstones and chronic cholecystitis are considered major risk factors. An association with ulcerative colitis has been suggested, but statistically not established [2]. Liver-fluke infections are known promoters of biliary adenocarcinomas, possibly in association with dietary nitrosamine uptake [3].

As effective chemotherapy is unavailable to date, any chance of a cure through adequate surgery depends on early detection.

To date, adenocarcinoma cell lines originating from the gallbladder or extrahepatic biliary tract have rarely been established in permanent tissue culture [4–6] and little is known about their biology or biochemical markers. Tumour cell lines in permanent tissue culture open possibilities to further define markers that may become clinically relevant [7–12].

Additionally, tissue culture cell lines facilitate studies on clonal tumour heterogeneity, and may thus add to a further understanding of the role of the immune response to cancers.

We report here on three newly established adenocarcinoma cell lines derived from two carcinomas of the gallbladder and from a primary carcinoma of the extrahepatic biliary tract, with emphasis on cell morphology, differentiation, chromosomal heterogeneity and biochemical markers.

Methods

Patients

Mz-ChA-1 was derived from an abdominal wall metastasis of a highly differentiated papillary adenocarcinoma of the gall bladder of a 55-year-old female patient. Ten years earlier the patient had been treated for cholecystitis. Five months before the tumour specimen was obtained for tissue culture, a cholecystectomy was performed because of symptomatic cholelithiasis. The surgical specimen showed the unexpected presence of a highly differentiated, mucus producing gallbladder adenocarcinoma. Subsequently, re-laparotomy became necessary due to obstructive

Material Safety Data Sheet

Version 3.1
Revision Date 10/22/2010
Print Date 05/18/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : γ-Aminobutyric acid

Product Number : A5835
Brand : Sigma
Product Use : For laboratory research purposes.

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

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

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

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Central nervous system

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects

Moderate skin irritant
Moderate respiratory irritant
Moderate eye irritant

GHS Classification

Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity - single exposure (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word Warning

Hazard statement(s)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HMIS Classification

Health hazard: 2
Chronic Health Hazard: *

Flammability: 0
Physical hazards: 0

Potential Health Effects

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

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 4-Aminobutanoic acid
Piperidic acid
Piperidinic acid
GABA

Formula : $C_4H_9NO_2$
Molecular Weight : 103.12 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
4-Aminobutyric acid			
56-12-2	200-258-6	-	-

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
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

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. - Carbon oxides, nitrogen oxides (NOx)

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
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.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. 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	colourless

Safety data

pH	6.0 - 8.0 at 103.1 g/l at 25 °C (77 °F)
Melting/freezing point	Melting point/range: 195 °C (383 °F) - dec.
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.103.1 g/l at 20 °C (68 °F)
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. - Carbon oxides, nitrogen oxides (NOx)

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - mouse - 12,680 mg/kg

Inhalation LC50
Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

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

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
Inhalation - May cause respiratory irritation.

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

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

Synergistic effects
no data available

Additional Information
RTECS: ES6300000

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.

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 listed on the Canadian NDSL list. All other components are on the Canadian DSL list.

	CAS-No.
4-Aminobutyric acid	56-12-2

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate respiratory irritant Moderate eye irritant

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

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.

Material Safety Data Sheet

Version 4.3
Revision Date 11/05/2011
Print Date 05/18/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Muscimol
Product Number	:	M1523
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

Central nervous system

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by ingestion

GHS Classification

Acute toxicity, Oral (Category 2)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H300	Fatal if swallowed.

Precautionary statement(s)

P264	Wash hands thoroughly after handling.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.

HMIS Classification

Health hazard:	3
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 fatal if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 3-Hydroxy-5-aminomethyl-isoxazole
5-Aminomethyl-3-isoxazolol
5-Aminomethyl-3-hydroxy-isoxazole

Formula : $C_4H_6N_2O_2$

Molecular Weight : 114.1 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
5-(Aminomethyl)isoxazol-3-ol hydrate			
2763-96-4	220-430-4	-	-

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. Take victim immediately to hospital. 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. FIREFIGHTING 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 firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. 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.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

Keep in a dry place.

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
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	white

Safety data

pH	no data available
Melting point/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	no data available
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, Strong acids, Acid chlorides, Acid anhydrides

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 45 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

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

no data available

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 fatal if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
Central nervous system depression, May cause convulsions., Symptoms may be delayed., Neurotoxic effects., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: NY3325000

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)
UN number: 1544 Class: 6.1 Packing group: II

Proper shipping name: Alkaloids, solid, n.o.s. (5-(Aminomethyl)isoxazol-3-ol hydrate)
Reportable Quantity (RQ): 1000 lbs
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 1544 Class: 6.1 Packing group: II EMS-No: F-A, S-A
Proper shipping name: ALKALOIDS, SOLID, N.O.S. (5-(Aminomethyl)isoxazol-3-ol hydrate)
Marine pollutant: No

IATA
UN number: 1544 Class: 6.1 Packing group: II
Proper shipping name: Alkaloids, solid, n.o.s. (5-(Aminomethyl)isoxazol-3-ol hydrate)

15. REGULATORY INFORMATION

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by ingestion

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

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.

Material Safety Data Sheet

Version 4.3
Revision Date 09/09/2011
Print Date 05/18/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Picrotoxin

Product Number : P1675
Brand : Sigma
Product Use : For laboratory research purposes.

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

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

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

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Central nervous system

WHMIS Classification

D1A Very Toxic Material Causing Immediate and Serious Toxic Effects — Highly toxic by ingestion

GHS Classification

Acute toxicity, Oral (Category 2)
Acute aquatic toxicity (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H300 Fatal if swallowed.
H402 Harmful to aquatic life.

Precautionary statement(s)
P264 Wash hands thoroughly after handling.
P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.

HMIS Classification

Health hazard: 3
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 fatal if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cocculin

Formula : $C_{15}H_{18}O_7 \cdot C_{15}H_{16}O_6$
Molecular Weight : 602.58 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Picrotoxin			
124-87-8	204-716-6	-	-

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. Take victim immediately to hospital. 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. - Carbon oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. 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. Discharge into the environment must be avoided.

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.

Reproductive toxicity

no data available

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 fatal if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
Convulsions, CNS stimulation., To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: TJ9100000

12. ECOLOGICAL INFORMATION

Toxicity

Toxicity to fish	LC50 - Gambusia affinis (Mosquito fish) - 8.36 mg/l - 48.0 h

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

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Harmful to aquatic life.

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)
UN number: 3462 Class: 6.1 Packing group: II
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Picrotoxin)
Reportable Quantity (RQ):
Marine pollutant: Marine pollutant
Poison Inhalation Hazard: No

IMDG
UN number: 3462 Class: 6.1 Packing group: II EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Picrotoxin)
Marine pollutant: Marine pollutant

IATA
UN number: 3462 Class: 6.1 Packing group: II
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Picrotoxin)

15. REGULATORY INFORMATION

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by ingestion

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

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:	gamma-Aminobutyric acid (GABA)
Proposed Use Dose:	3000 µg
Proposed Storage Dose:	10000000 µg
LD_{50} (species):	12680000 µg

Calculation:

12680000 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 634000000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 63400000

Comments/Recommendations:

Western
UNIVERSITY · CANADA

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Muscimol
Proposed Use Dose:	24 µg
Proposed Storage Dose:	10000 µg
LD_{50} (species):	45000 µg

Calculation:

45000 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 2250000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 225000

Comments/Recommendations:

Western
UNIVERSITY · CANADA

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Picrotoxin
Proposed Use Dose:	2 µg
Proposed Storage Dose:	**5000000** µg
LD $_{50}$ (species):	15000 µg

Calculation:

15000 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 750000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = ***75000***

Comments/Recommendations: