Modification Form for Permit BIO-UWO-0141

Permit Holder: Sashko Damjanovski

Approved Personnel
(Please stroke out any personnel to be removed)

Michelle Niewansteeg

Logan Walsh

Max Shafer

Mark Fox

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DHS - alpha	
Approved Cells	human (established) Hs 578 BST	MCF10A CELLS ATCC # CRL 10317
Approved Use of Human Source Material	human (established): MDA MB 231, frog (established) A6	
Approved GMO		
Approved use of Animals	Xenopus laevis	
Approved Toxin(s)		CHOLERA TOXIN SIGMA CAT # C8052

Description of Work to be done with with additional agents

1. MCF 10A cells

The MCF 10A cell line is a non-tumorigenic epithelial cell line to be used as a control in conjunction with our already approved cell lines MDA MB 231 and Hs578t.

2. Cholera Toxin (LD50 250μg/kg)

Cholera Toxin is required for growth of the MCF10 cell line as described by the ATCC:

Propagation of MCF10A cells
The base medium for this cell line is MEBM, which is supplied as part of the MEGM Bullet Kit available from Clonetics Corporation, Catalog No. CC-3150. To make the complete growth medium, add the following components to the base medium: All MEGM SingleQuot additives that are supplied with the kit except the GA-1000 (BPE 13 mg/ml, 2 ml; hydrocortisone 0.5 mg/ml, 0.5 ml; hEGF 10 ug/ml, 0.5 ml; insulin 5 mg/ml, 0.5 ml); **100 ng/ml cholera toxin (sold separately). Note: 0.5MG was ordered.**
Temperature: 37.0°C

Cholera Toxin will be stored in a locked cabinet in WSC 357G2 – A room with restricted access (key-card only).

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http:// www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 1

Date of Last Biohazardous Agents Registry Form: Mar 28, 2008

Date of Last Modification (if applicable): Jun 26, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I
Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
Physical data

Pink or red aqueous liquid

SECTION III
Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV
Fire and explosion

Not applicable

SECTION V
Reactivity data

Stable. Hazardous polymerization will not occur.

SECTION VI
Method of disposal

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.
Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII
Special protection information

For Biosafety Level 1 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII
Special precautions or comments

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

February 2002

MATERIAL SAFETY DATA SHEET

Date Printed: 11/11/2009
Date Updated: 07/20/2009
Version 1.10

Section 1 - Product and Company Information

Product Name	CHOLERA TOXIN FROM VIBRIO CHOLERAE
Product Number	C8052
Brand	SIGMA
Company	Sigma-Aldrich Canada, Ltd
Address	2149 Winston Park Drive Oakville ON L6H 6J8 CA
Technical Phone:	9058299500
Fax:	9058299292
Emergency Phone:	800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #		SARA 313
CHOLERA TOXIN	9012-63-9		No
Ingredient Name	**CAS #**	**Percent**	**SARA 313**
TRIS (HYDROXYMETHYL) AMINOMETHANE FREE BASE	77-86-1	>= 5.82 <= 5.94	No
TRIS (HYDROXYMETHYL) AMINOMETHANE HYDROCHLORIDE SALT	1185-53-1	>= 31.3 <= 31.9	No
ETHYLENEDIAMINETETRAACETIC ACID DISODIUM DIHYDRATE	6381-92-6	>= 1.83 <= 1.87	No
SODIUM AZIDE	26628-22-8	>= 0.96 <= 0.98	Yes
SODIUM CHLORIDE	7647-14-5	>= 57.6 <= 58.8	No
CHOLERA TOXIN FROM VIBRIO CHOLERAE	9012-63-9	>= 0.5 <= 2.5	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Harmful.
Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

HMIS RATING

HEALTH: 2*
FLAMMABILITY: 0
REACTIVITY: 1

NFPA RATING

HEALTH: 2
FLAMMABILITY: 0
REACTIVITY: 1

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

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

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

Section 5 - Fire Fighting Measures

EXPLOSION HAZARDS
Azide reacts with many heavy metals such as lead, copper, mercury, silver, gold to form explosive compounds. Copper and lead azides are more sensitive than nitroglycerine. Azide reacts with metal halides to give a range of metal azide halides, many of which are explosive. Incompatible with chromyl chloride, hydrazine, bromine, carbon disulfide, dimethyl sulfate, dibromomalonitrile.

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

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

METHODS FOR CLEANING UP
Spilled material should be carefully wiped up or moistened with water and removed. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING

User Exposure: Do not use if skin is cut or scratched. Wash thoroughly after handling. Container should be opened only by a technically qualified person. Handle as if capable of transmitting infectious agents.

STORAGE

Suitable: Keep tightly closed.
Store at 2-8°C

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face 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.
Hand: Compatible chemical-resistant gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES

Wash thoroughly after handling. Wash contaminated clothing before reuse.

Section 9 - Physical/Chemical Properties

Appearance — Physical State: Solid

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	

Miscellaneous Data	N/A
Solubility	N/A

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.
Materials to Avoid: Dimethyl sulfate is incompatible with sodium azid, Acid chlorides, Halogenated solvents Avoid contact with metals. Avoid contact with acid. Azide reacts with many heavy metals such as lead, copper, mercury, silver, gold to form explosive compounds. Copper and lead azides are more sensitive than nitroglycerine. Azide reacts with metal halides to give a range of metal azide halides, many of which are explosive. Incompatible with chromyl chloride, hydrazine, bromine, carbon disulfide, dimethyl sulfate, dibromomalonitrile.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: Causes skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: Causes eye irritation.
Inhalation: May be harmful if inhaled. Material is irritating to mucous membranes and upper respiratory tract.
Ingestion: Harmful if swallowed.

SIGNS AND SYMPTOMS OF EXPOSURE

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated. Diarrhea. Nausea, headache, and vomiting. Many azides cause a fall in blood pressure and some inhibit enzyme action. Unlike A subunit and cholera toxin, the B subunit is reported to be non-toxic. Cholera toxin acts in the lumen of the small intestine of man to produce a massive outpouring of small intestinal secretion resulting in a loss of body water and electrolytes. Recovery is the rule following replacement of water and electrolytes.(ref.: J. Of Infectious Diseases vol. 133 supplement, p. S31, 1976) Laboratory experiments in animals have shown sodium azide to produce a profound hypotensive effect, demyelination of myelinated nerve fibers in the central nervous system, testicular damage, blindness, attacks of rigidity, and hepatic and cerebral effects. Exposure can cause:

CONDITIONS AGGRAVATED BY EXPOSURE

Exposure to cholera toxin can cause a massive loss of water and electrolytes from the intestine.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Observe all federal, state, and local environmental regulations. Cholera Toxin may be deactivated by one of three methods: 1) Boil at 100°C for 30 minutes. 2) Autoclave at 121°C and 15 psi for 30 minutes. 3) Exposure to acidic conditions (0.1N HCl).

Section 14 - Transport Information

DOT
Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

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

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: Xn
Indication of Danger: Harmful.
R: 22-36/37/38-52/53
Risk Statements: Harmful if swallowed. Irritating to eyes, respiratory system and skin. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S: 26-60
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. This material and its container must be disposed of as hazardous waste.

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Harmful.
Risk Statements: Harmful if swallowed. Irritating to eyes, respiratory system and skin.
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. This material and its container must be disposed of as hazardous waste.
US Statements: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Modification Form for Permit BIO-UWO-0141

Permit Holder: Sashko Damjanovski

Approved Personnel
(Please stroke out any personnel to be removed)

Michelle Niewansteeg

Logan Walsh

Additional Personnel
(Please list additional personnel here)

MAX SHAFER (SEPT 09 START)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DHS - alpha	
Approved Cells	human (established) Hs 578 BST	hamgn (established) MDA MB 231 ~~MDA MB 435~~ removed - see e-mail attached V. frog (established) A6
Approved Use of Human Source Material		
Approved GMO		
Approved use of Animals	Xenopus laevis	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 1

Date of last Biohazardous Agents Registry Form: Mar 28, 2008

Signature of Permit Holder: [signature]

BioSafety Officer(s): [signature] June 26/09

Chair,Biohazards Subcommittee: [signature]

Modification Form for Permit BIO-UWO-0141

Permit Holder: Sashko Damjanovski

Approved Toxin(s)

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 1

Date of last Biohazardous Agents Registry Form: Mar 28, 2008

Signature of Permit Holder:

BioSafety Officer(s): June 26/09

Chair,Biohazards Subcommittee:

June 23, 2009

We wish to begin to use 3 new cell lines in our research.

The MDA MB 231 cell line, and its derivative MDA MB 435 line, are established human epithelial breast adenocarcinoma cell lines that are available from ATCC. Both cell types proliferate relatively quickly and have migratory capabilities, however only the 231 line, and not the 435 has invasive capabilities. Thus when plated on a 3D extracellular matrix substrate *in vitro* (such as collagen, or commercially available matrigel) the 231 cells can invade the matrix, while the 435 cannot. In addition the *Xenopus laevis* (frog) established epithelial cell line A6, (also from ATCC) will also be used in our migration studies. As our lab studies the functions of matrix metalloproteinases, secreted enzymes that are in part responsible for the cleavage and remodelling of extracellular matrices, we will use these like to look more specifically at the function of our specific matrix metalloproteinase through the transient transfection of our genes into these cells and examine their subsequent invasive capabilities. Work with these cells will all be *in vitro* and performed in the certified tissue culture room in the molecular genetics core in the WSC building (not on out lab in BGS).

Thus in addition to the cell line we are using HS 578 BST, we wish to add
human MDA MB 231 and
human MDA MB 435
frog A6

Thanks

Sashko Damjanovski

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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

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Cell Biology

ATCC® Number: HTB-26™ Order this Item

Designations: MDA-MB-231

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $256.00

Depositors: R Cailleau

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

PHOTO

Source:
Organ: mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

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.

Related Cell Culture Products

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Receptors: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

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

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Cell Biology

ATCC® Number: HTB-129™ Order this Item

Price: $264.00

Designations: MDA-MB-435S

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: spindle shaped

PHOTO

Source: **Organ:** previously described as: mammary gland; breast
Disease: previously described as ductal carcinoma
Derived from metastatic site: pleural effusion

Cellular Products: tubulin; actin

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** 1976

Tumorigenic: No

DNA Profile (STR): Amelogenin: X
CSF1PO: 11
D13S317: 12
D16S539: 13
D5S818: 12
D7S820: 8,10
THO1: 6,7
TPOX: 8,11
vWA: 16,18

Cytogenetic Analysis: modal number = 56; range = 55 to 62
The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
PGM1, 2
PGM3, 1

Age: 31 years adult

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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

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Cell Biology

ATCC® Number:	CCL-102™ Order this Item	**Price:**	$323.00
Designations:	A6	**Depositors:**	KA Rafferty
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	Xenopus laevis (frog, South African clawed)	**Morphology:**	epithelial

Source: **Organ:** kidney
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.

Related Cell Culture Products

Virus Susceptibility: FV-4 (Frog virus)

Virus Resistance: poliovirus 1; vesicular stomatitis (Indiana); herpes simplex; vaccinia; pseudorabies

Reverse Transcript: negative

Age: adult

Gender: male

Propagation: **ATCC complete growth medium:** NCTC 109 medium, 75%; distilled water, 15%; fetal bovine serum, 10%
Temperature: 26.0°C

Subculturing: **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: Twice per week
Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach.
Add fresh culture medium, aspirate and dispense into new culture flasks.

Preservation: culture medium 95%; DMSO, 5%

Related Products: recommended serum:ATCC 30-2020

References: 21414: . Biology of amphibian tumors. New York: Springer-Verlag; 1969.
33005: Rokaw MD, et al. Regulation of a sodium channel-associated G-protein by aldosterone. J. Biol. Chem. 271: 4491-4496, 1996. PubMed: 8626803

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BIO-UWO-0141

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: September, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR SASHKO DAMJANOVSKI
SIGNATURE
DEPARTMENT BIOLOGY
ADDRESS 1151 RICHMOND ST LONDON ON
PHONE NUMBER (519) 661-2111 x 84704
EMAIL sdamjano@uwo.ca

Location of experimental work to be carried out: Building(s) BGS WSC Room(s) 318 328 (Being Renovated)

*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

GRANT TITLE(S): MMP ACTIVATION DURING XENOPUS DEVELOPMENT

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NSERC

Names of all personnel working under Principal Investigators super vision in this location:

LOGAN WALSH
MICHELLE Niewanstecg

DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒ YES ○ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?	Source/ Supplier	Health Canada or CFIA Containment Level
Bacteria	☒ Yes ☒ No	☒ Yes ☒ No	○ Yes ☒ No	50mL	Invitrogen	☒ 1 ○ 2 ○ 3
(DH5α)	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	○ Yes ○ No	
Rodent	○ Yes ○ No	
Non-human primate	○ Yes ○ No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	☒ Yes ○ No	Hs 578Bst	ATCC # HTB 125
Rodent	○ Yes ○ No		
Non-human primate	○ Yes ○ No		
Other (specify)	○ Yes ○ No		

2.4 For above named cell types(s) indicate HC or CFIA containment level required ☒ 1 ○ 2 ○ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES Ø 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 Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs (preserved)		O Yes O No		O 1 O 2 O 3

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

4.2 Will genetic sequences from the following be involved:
- HIV O YES Ø NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens O YES Ø NO
if YES specify_____
- Other human or animal pathogen and or their toxins O YES Ø NO
if YES specify_____

4.3 Will intact genetic sequences be used from
- SV 40 Large T antigen O YES Ø NO If YES specify_____
- Known oncogenes O YES Ø NO If YES specify_____

4.4 Will a live viral vector(s) or bacterial plasmid be used for gene transduction O YES Ø NO
If YES name _____.
Please attach a Material Safety Data Sheet or equivalent.

4.5 List specific vector(s) to be used: _____

4.6 Will virus be replication defective O YES Ø NO

4.7 Will virus be infectious to humans or animals O YES Ø NO

4.8 Will this be expected to increase the Containment Level required O YES Ø NO

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5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? O YES ⊗ NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES ⊗ NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? O YES ⊗ NO (NA) O PENDING

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? O YES O NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used Xenopus laevis

6.3 AUS protocol # 2005-027-05

6.4 If using murine cell lines, have they been tested for murine pathogens? ✓ YES ✓ NO N/A

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:

- Pound source dogs O YES ⊗ NO
- Pound source cats O YES ⊗ NO
- Cattle, sheep or goats O YES ⊗ NO
- Non- Human Primates O YES ⊗ NO If YES specify species _____
- Wild caught animals O YES ⊗ NO If YES specify species _____ colony # _____
- Birds O YES ⊗ NO
- Others (wild or domestic) O YES ⊗ NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin_____

8.3 What is the LD_{50} (specify species) of the toxin_____

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

9.0 Import Requirements

9.1 Will the agent be imported? O YES ☒ NO
If no, please proceed to Section 10.0
If yes, country of origin_____

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

9.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O NO

9.4 Has the import permit been sent to OHS? O YES O NO
If yes, Permit # _____

10.0 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
- Employee Health and Safety Orientatioin

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE J. Dayanandan

11.0 Containment Levels

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

11.2 Has the facility been certified by OHS for this level of containment? ☒ YES O NO

11.3 If yes, please give the date and permit number: _____

BG 5318 closed for renovations until 2009. sharing WSC 323 with Dr Kohalmi under her level 1 permit.

12.0 Approvals

UWO Biohazard Subcommittee.

Signature G.M. Kidder Date 28 Mar. '08

Safety Officer for Institution where experiments will take place

Signature S. Stanley, UWO Date March 28, 2008

Safety Officer for University of Western Ontario (if different from above)

Signature_____ Date_____

Expiry Date (3 years from Approval): _____

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