Modification Form for Permit BIO-RR1-0022

Permit Holder: R. Rylett

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

Kirk Young

Ewa Jaworski

Elizabeth Banasikowska

~~Alexie Gordon~~

Ventzi Hristova

Fatima Abji

Stefanie Black

Kathy James

Daisy Wong

Additional Personnel
(Please list additional personnel here)

Leah Cuddy MSc (started Sept 2009)

Rachel Mixer PhD (")

* *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: 2

Date of last Biohazardous Agents Registry Form: Aug 4, 2009

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-RRI-0022

Permit Holder: R Ryley

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli dh5 alpha	Clostridium Difficile Toxin B
Approved Cells	Human (established) - HEK 293, SH-Sy5Y. Rodent (established), , PC12,	Botulinum Toxin C from clostridium
Approved Use of Human Source Material		
Approved GMO	[Plasmid] - pcDNA 3.1.	
Approved use of Animals		
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: 2

Date of last Biohazardous Agents Registry Form: Aug 4, 2009

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

DESCRIPTION OF BIOHAZARDS AND HOW THEY WILL BE USED

Clostridium difficile toxin B irreversibly glucosylates members of the Rho family of GTPases, small proteins involved in the regulation of actin dynamics, the endocytosis of proteins and membrane lipids. Cells in culture will be treated *in vitro* with *C. difficile* toxin B at a concentration of 1 ug/mL to determine the effect of Rho GTPases upon protein endocytosis.

MATERIAL SAFETY DATA SHEET

Date Printed: 08/24/2009
Date Updated: 01/26/2006
Version 1.3

Section 1 - Product and Company Information

Product Name	CLOSTRIDUM DIFFICILE TOXIN B
Product Number	C4102
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
CLOSTRIDIUM DIFFICILE TOXIN B	None	No

Chemical Family Etiological agent.
Synonyms Clostridium difficile toxin B * Toxb-Dif
RTECS Number: XW5807300

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Biohazard. Irritant.
Irritating to eyes, respiratory system and skin.
Biomedical material. May cause human disease. Poison. May be fatal if enters bloodstream. Do not breathe dust. Do not use if skin is cut or scratched. Wash thoroughly after handling.

HMIS RATING

HEALTH: 4
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING

HEALTH: 4
FLAMMABILITY: 0
REACTIVITY: 0

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.

INFORMATION FOR PHYSICIAN
Advise the physician of the compound to which the person was exposed.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

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

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

Section 6 - Accidental Release Measures

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

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear self-contained breathing apparatus, 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 pipet by mouth.

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

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Use only in a chemical fume hood.

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 contaminated clothing before reuse.

Section 9 - Physical/Chemical Properties

Appearance — Physical State: Liquid

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

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: Causes skin irritation.
Eye Contact: Causes eye irritation.
Inhalation: Material is irritating to mucous membranes and upper respiratory tract.
Multiple Routes: May be harmful by inhalation, ingestion, or skin absorption.

SENSITIZATION
Sensitization: Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

TARGET ORGAN(S) OR SYSTEM(S)
G.I. System.

SIGNS AND SYMPTOMS OF EXPOSURE
May be fatal if enters bloodstream. May cause nausea, vomiting, weakness, gastrointestinal disorders, disturbances of electrolyte balance, hypotension, depressed respiration.

CONDITIONS AGGRAVATED BY EXPOSURE
May be fatal if enters bloodstream.

TOXICITY DATA

Intravenous
Mouse
>200 MG/KG
LD50

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.

Section 14 - Transport Information

DOT
Proper Shipping Name: Toxins, extracted from living sources, solid, n.o.s.
UN#: 3462
Class: 6.1
Packing Group: Packing Group I
Hazard Label: Toxic substances.
PIH: Not PIH

IATA
Proper Shipping Name: Toxins, extracted from living sources, solid, n.o.s.
IATA UN Number: 3462
Hazard Class: 6.1
Packing Group: I

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: Xi
Indication of Danger: Irritant.
R: 36/37/38
Risk Statements: Irritating to eyes, respiratory system and skin.
S: 26-36
Safety Statements: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Biohazard. Irritant.
Risk Statements: 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.
US Statements: Biomedical material. May cause human disease. Poison. May be fatal if enters bloodstream. Do not breathe dust. Do not use if skin is cut or scratched. Wash thoroughly after handling.

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

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

Section 16 - Other Information

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

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

The botulinum neurotoxin C will be used to impare synaptic vesicle exocytosis in experiments to test if the sodium-dependent choline transporter recycles via synaptic vesicles following activation of protein kinase C.

MATERIAL SAFETY DATA SHEET

Date Printed: 08/24/2009
Date Updated: 02/02/2006
Version 1.5

Section 1 - Product and Company Information

Product Name	BOTULINUM TOXIN C FROM CLOSTRIDIUM &
Product Number	B1036
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
BOTULINUM TOXIN C FROM CLOSTRIDIUMBOTULINUM	93384-45-3		No
Ingredient Name	**CAS #**	**Percent**	**SARA 313**
BOTULINUM TOXIN SUBUNIT	None	0.1 <= 0.1	
SODIUM CHLORIDE	7647-14-5	1.17 <= 1.17	No
SODIUM ACETATE, ANHYDROUS	127-09-3	0.41 <= 0.41	No
WATER	7732-18-5	<= 98.32	No

Chemical Family Etiological agent.
Synonyms BOTULIN NEUROTOXIN C * BORALIN TOXIN C * TOXIN BOTULIN C

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Biohazard. Highly Toxic (USA) Very Toxic (EU).
Very toxic by inhalation, in contact with skin and if swallowed.
Irritating to eyes and skin.
Biomedical material. May cause human disease. Target organ(s): Nerves.

HMIS RATING

HEALTH: 4*
FLAMMABILITY: 0
REACTIVITY: 1

NFPA RATING

HEALTH: 4
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.

INFORMATION FOR PHYSICIAN
In certain emergency situations, the Center for Disease Control can supply botulinum toxoid, pentavalent (abcde) an investigational new drug. These biological products are available through the Immunobiologics Bureau of Laboratories, Center for Disease Control in Atlanta, Georgia USA. Phone requests may be made to CDC at USA. 404-639-3311 from 8:00 am to 4:30 PM Monday through Friday. After working hours or on weekends and holidays call USA 404-639-2888. Advise the physician of the compound to which the person was exposed.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

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

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

Section 6 - Accidental Release Measures

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

PROCEDURE(S) OF PERSONAL PRECAUTION(S)
Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP
Absorb on sand or vermiculite and place in closed containers for disposal. Wash spill site with soap solution.

Section 7 - Handling and Storage

HANDLING
User Exposure: Botulinum toxin a should be handled in a closed system. All operations should be carried out in a glove bag of similar enclosure to avoid accidental contact. 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 -20°C

SPECIAL REQUIREMENTS
Light sensitive. Heat sensitive.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Use only in a chemical fume hood. Safety shower and eye bath.

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 contaminated clothing before reuse. Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance	Physical State: Liquid	
Property	Value	At Temperature or Pressure
Molecular Weight	350,000 AMU	
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.
Conditions to Avoid: Direct sunlight Heat.
Materials to Avoid: Bases.

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

SIGNS AND SYMPTOMS OF EXPOSURE
This toxin is among the most powerful paralytic poisons known, having irreversible effects. Considered a lethal neurotoxin, 1ug may be fatal if swallowed or inhaled. Orl-man LD50:1ug/man (Microbial Toxins, Vol. IIa,1971). Botulinum toxin acts principally by paralyzing the synapses (junctions) of the peripheral nerves leading to muscles. Physiological changes include nausea, vertigo, pharyngeal pain, blurred vision, constipation, and respiratory paralysis. Type E toxin acts more slowly than type A, which has a rapid time of onset. After ingestion of a sufficient dose of type E toxin, humans show symptoms in 4 to 6 hours, and death occurs in several days. (Frontier 26, (2), 17 ,1965).

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
After use, utensils and the toxin solution should be soaked overnight or boiled for 20 minutes in an excess amount of 2% sodium hydroxide solution or sodium hypochlorite (1% available

chlorine) solution in a hood or well ventilated area. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: Toxins, from living sources, liquid, n.o.s.
UN#: 3172
Class: 6.1
Packing Group: Packing Group I
Hazard Label: Toxic substances.
PIH: Not PIH

IATA
Proper Shipping Name: Toxins, extracted from living sources, liquid, n.o.s.
IATA UN Number: 3172
Hazard Class: 6.1
Packing Group: I

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION
Symbol of Danger: B-T+
Indication of Danger: Biohazard. Very toxic.
R: 26/27/28-36/38
Risk Statements: Very toxic by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin.
S: 53-45
Safety Statements: Restricted to professional users. Attention - Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

US CLASSIFICATION AND LABEL TEXT
Indication of Danger: Biohazard. Highly Toxic (USA) Very Toxic (EU).
Risk Statements: Very toxic by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin.
Safety Statements: Restricted to professional users. Attention - Avoid exposure - obtain special instructions before use. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
US Statements: Biomedical material. May cause human disease.
Target organ(s): Nerves.

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.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 ~~also~~ be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Rebecca Jane Rylett
SIGNATURE	
DEPARTMENT	Molecular Brain Research Group
ADDRESS	Robarts Research Institute
PHONE NUMBER	519-931-5777 ext 24078
EMERGENCY PHONE NUMBER(S)	519-931-5777
EMAIL	jane.rylett@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Robarts Institute Room(s) 3rd floor

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Regulation of choline acetyltransferase at the cholinergic neuron

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

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

Ewa Jaworski
Daisy Wong
Kathy James
Stefanie Black
Fatima Abji
Kirk Young
Ventzi Hristova
Alexis Gordon
Elizabeth Banasikowska

*** 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 O NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli dh5alpha	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	250 mL	See 4.2	⊗ 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

See attached e-mail ql.

*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 O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ⊗ No		Not applicable
Rodent	O Yes ⊗ No		
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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 O No	HEK 293, SH-SY5Y	ATCC
Rodent	⊗ Yes O No	PC12	ATCC
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O 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 Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O 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 or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (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? ⊗ YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5alpha	pcDNA3.1	Clontech	choline acetyltransferase	. no apparent change except expression of protein

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

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ⊘ NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? 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 O 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, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body 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, please specify species _____ ⊗ NO
- Wild caught animals O YES, please specify species & colony # _____ ⊗ NO
- Birds O YES ⊗ NO
- Others (wild or domestic) O YES, please specify _____ ⊗ 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(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

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

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

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 10.0 Ø NO

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

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

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

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

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

SIGNATURE _____

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

13.0 Containment Levels

13.1 For the work described in sections 9.0 to 9.0 please indicate the highest HBC or CFIA Containment Level required: ○ 1 ○ 2 ○ 3

13.2 Has the facility been certified by OBS for this level of containment?
- ○ YES, permit # if on campus: _____
- ○ NO, please certify
- ○ NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.uwo.ca/

SIGNATURE: _____ Date: _____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: B10-RR1-0022 Expiry Date (3 years from Approval): August 4, 2012

Special Conditions of Approval:

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

Hello Jennifer

Here is a statement about our work:

Studies are focussed on changes in brain chemistry associated with normal aging and degenerative neurological and psychiatric diseases. This provides an assessment of how nerve cells communicate and conditions that promote healthy brain aging, and therapeutic interventions that may be beneficial for treatment of dusfunction. Experimental models involve cellular and molecular approaches, protein chemistry and function, trafficking of proteins in cells and interactions of cellular constituents and their role in regulation of cell function.

I hope that this helps
Jane Rylett

------- Original Message --------

Subject: Re: Biohazardous Agents Registry Form: Rylett
Date: Tue, 14 Jul 2009 09:40:13 -0400
From: Jane Rylett <jane.rylett@schulich.uwo.ca>
To: jstanle2@uwo.ca
References: <4A5C527D020000C800018EB0@draco.med.uwo.ca>
<4A5C527D020000C800018EB3@draco.med.uwo.ca>

Yes that is correct. We normally do 100 ml or 250 ml cultures

Jane Rylett

-----Original Message-----
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Rylett, Jane <Jane.Rylett@schulich.uwo.ca>

Sent: 7/14/2009 9:38:35 AM
Subject: Biohazardous Agents Registry Form: Rylett

Thanks Dr. Rylett:
I noticed you said "yes" to question 1.1 (the use of micoorganisms or biological agents). However, Table 1.2 was not completed. I suspect that the only micoorganism that you use is E.coli dh5 alpha (less than 1 litre of it cultured at one time)...can you confirm this?
- Jennifer

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Cell Biology

ATCC® Number: CRL-1573™ Order this Item

Designations: 293 [HEK-293]

Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $256.00

Depositors: FL Graham

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

Source: **Organ:** embryonic kidney
Cell Type: transformed with adenovirus 5 DNA

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

Restrictions: These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications: efficacy testing [92587]
transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
viruscide testing [92579]

Receptors: vitronectin, expressed

Tumorigenic: Yes

DNA Profile (STR): Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments: Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]

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Cell Biology

ATCC® Number:	**CRL-2266™** Order this Item	**Price:**	**$264.00**
Designations:	SH-SY5Y	**Depositors:**	JL Biedler
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	mixed, adherent and suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
			PHOTO

Source: **Organ:** brain
Disease: neuroblastoma
Derived from metastatic site: bone marrow

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

Restrictions: NOTE: SH-SY5Y was deposited at the ATCC by June L. Biedler, Memorial Sloan-Kettering Cancer Center. SH-SY5Y is distributed for academic research purposes only. Memorial Sloan-Kettering releases the line subject to the following: 1.) SH-SY5Y or its products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of SH-SY5Y including any use by a for-profit entity must first be negotiated with Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1970

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

Antigen Expression: Blood Type A; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 11
D16S539: 8,13
D5S818: 12
D7S820: 7,10
THO1: 7,10
TPOX: 8,11
vWA: 14,18

Cytogenetic Analysis: modal number = 47; the cells possess a unique marker comprised of a chromosome 1 with a complex insertion of an additional copy of a 1q segment into the long arm, resulting in trisomy of 1q [22554]

Age: 4 years

Gender: female

Comments: SH-SY5Y cells have a reported saturation density greater than 1 X 10(6) cells/sq cm. They are reported to exhibit moderate levels of dopamine beta hydroxylase activity [PubMed ID: 29704].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is a 1:1 mixture of ATCC-formulated

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Cell Biology

ATCC® Number: CRL-1721™ Order this Item

Price: $256.00

Additional Information about this cell line

Designations: PC-12

Depositors: B Patterson

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: loosely adherent, multicell aggregates

Organism: Rattus norvegicus (rat)

Morphology: polygonal

Source: **Organ:** adrenal gland
Disease: pheochromocytoma

Cellular Products: catecholamines; dopamine; norepinephrine [1163]

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 (Roche FuGENE® Transfection Reagents technology from amaxa)

Receptors: nerve growth factor (NGF), expressed

Tumorigenic: Yes

Cytogenetic Analysis: 40 chromosomes; 38 autosomes plus XY [1163]

Gender: male

Comments: The PC-12 cell line was derived from a transplantable rat pheochromocytoma. [1163]
The cells respond reversibly to NGF by induction of the neuronal phenotype. [1163]
The cells do not synthesize epinephrine. [1163]

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 2.5%; horse serum to a final concentration of 15%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.1. Remove and discard old culture medium.2. Pipet 10 ml fresh medium over the cell sheet and scrape.3. Aspirate cells with a small bore pipette to break up clusters.4. Add appropriate aliquots of the cell suspension to new 75 cm2 flask with 15 ml fresh growth medium. Seed flask at 1.0 x 10(4) to 3.0x 10(4) viable cells / cm2.Or use subcultivation ratio of 1:3 twice weekly Subculture when cell density reaches between 1.0x 1 0(5) to 2.0x 10(5) viable cells / cm2.5. Place culture vessels in incubator at 37°C.PC-12 cells adhere poorly to plastic and tend to grow in small patches of loosely attached cells.Attachment can be enhanced by coating the flasks with Bovine Collagen I or using Corning® CellBIND® Surface Flasks (Free Samples)
Subcultivation Ratio: 1:3 twice weekly