Modification Form for Permit BIO-RRI-0022

Permit Holder: R. Rylett

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

~~Kirk Young~~

Rachel Mixer

Leah Cuddy

~~Elizabeth Banasikowska~~

~~Ventzi Hristova~~

Fatima Abji

~~Stefanie Black~~

Kathy James

Daisy Wong

Ewa Jaworski

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms	E. coli dh5 alpha	5 Adenovirus.
Approved Primary and Established Cells	Human (established) - HEK 293, SH-Sy5Y. Rodent (established), , PC12,	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] - pcDNA 3.1.	
Approved Use of Animals		
Approved Biological Toxin(s)	Clostridium difficile toxin B, Botulinum neurotoxin C	

* *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 STORED, USED AND DISPOSED OF..*

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: R Dane RL MA

Current Classification: 2 **Containment Level for Added Biohazards:** _____

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

Date of Last Modification (if applicable): Oct 9, 2009

BioSafety Officer(s): Ronald Moseworthy May 02, 2011

Chair, Biohazards Subcommittee: _____ **Date:** _____

Attachment for the "5 Adenovirus"

The adenovirus is called "type 5 adenovirus." Here is a brief description of how it is used:

Recombinant adenoviral vectors are used for expression of proteins in primary neuron cultures and in human and/or rodent cell lines.
The adenovirus is added directly to culture medium and incubated for 48 hours.
For long-term storage, the adenovirus is kept at -80°C.
All serological pipettes, pipette tips and plasticware that come into contact with the adenovirus are decontaminated in 10% bleach for a minimum of 30 minutes.

Gene - choline acetyltransferase

Adenovirus [2]

Virology: Adenoviruses are medium-sized (90-100 nm), nonenveloped icosohedral viruses containing double-stranded DNA. There are 49 immunologically distinct types (6 subgenera: A through F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body.

The adenovirus infection cycle can be clearly divided into two phases, which are separated by viral DNA replication. The first or "early" phase covers the entry of the virus into the host cell and the entry of the virus genome to the nucleus. The late genes are transcribed from the major late promoter. The "late" phase is involved in making gene products that are related to production and assembly of capsid proteins.

Adenoviral Genes	Function
Early genes (E): E1A, E1B, E2, E3, E4	Adenoviral gene transcription, replication, host immune suppression, inhibition of host cell apoptosis
Delayed early genes: IX, IVa2	Packaging
Major late Unit (L)	Assembly

Virus packaged by transfecting HEK 293 cells with adenoviral-based vectors are capable of infecting human cells. These viral supernatants could, depending on the gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus.

The probability of producing replication competent adenovirus (RCA), although low, Increases with each successive amplification. RCA is produced when adenoviral DNA recombines with E1-containing genomic DNA in HEK 293 cells. It is suggested that to use early amplification stocks when needed to produce additional quantities of adenovirus.

Clinical features: Adenoviruses most commonly cause respiratory illness; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection. Acute respiratory disease (ARD) can be caused by adenovirus infections..

Epidemiology: Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, fecal-oral transmission, and occasionally waterborne transmission. Some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts, and shedding can occur for months or years. Some adenoviruses (e.g., serotypes 1, 2, 5, and 6) have been shown to be endemic in parts of the world where they have been studied, and infection is usually acquired during childhood. Other types cause sporadic infection and occasional outbreaks; for example, epidemic keratoconjunctivitis is associated with adenovirus serotypes 8, 19, and 37. Epidemics of febrile disease with conjunctivitis are associated with waterborne transmission of some adenovirus types. ARD is most often associated with adenovirus types 4 and 7 in the United States. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. For some adenovirus serotypes, the clinical spectrum of disease associated with infection varies depending on the site of infection; for example, infection with adenovirus 7 acquired by inhalation is associated with severe lower

respiratory tract disease, whereas oral transmission of the virus typically causes no or mild disease.

Treatment: Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

Laboratory hazards: Ingestion; droplet exposure of the mucous membrane

Laboratory Hazards	PPE
Exposure of mucus membrane (eyes, nose, mouth)	Use of safety goggles or full face shields. Use of appropriate face mask
Injection	Use of safety needles; NEVER re-cap needle or remove needle from syringe
Aerosole inhalation	Use of appropriate respiratory protection
Direct contact with skin	Gloves, lab coat, closed shoes

The above PPE are often required IN ADDITION to working in a certified Biosafety Cabinet.

Use with Animals: BSL-2 housing post injection/exposure of animals.

Adenovirus MSDS

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

Follow the "Biosecurity Requirements for Facilities Using Biological Agents" attached. Note the maximum quantities allowed. Fumehood must be used.

* *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: R Dave Rylett

BioSafety Officer(s): Stanley Oct 9/09

Chair,Biohazards Subcommittee: E.M. Kidder

Modification Form for Permit BIO-RRI-0022

Permit Holder: R. Rylett

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

Kirk Young

Ewa Jaworski

Elizabeth Banasikowska

~~Alexis Gordon~~

Ventzi Hristova

Fatima Abji

Stefanie Black

Kathy James

Daisy Wong

Additional Personnel
(Please list additional personnel here)

Leah Cuddy MSc (start at Sept 2009)

Rachel Mixer PhD (")

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Classification: 2

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

Signature of Permit Holder: Renee Rylett

BioSafety Officer(s): Q Stanley Oct 9/09

Chair,Biohazards Subcommittee: G M. Kidder

ATTACHMENT:
DESCRIPTION OF BIOHAZARDS AND HOW THEY WILL BE USED

Clostridium difficile toxin B

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

*Botulinum neuro*toxin C

*Botulinum neuro*toxin C will be used to impare synaptice vesicle exocytosis in experiments to test if the sodium-dependent choline transporter recycles via synaptice vesicles following activation of protein kinase C.

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

max amount allowed on hand is one order:
(1 ug/mL each time)

C. difficile 2ug

Botulium neurotoxin 0.1 mg (12 mL of 100ng mL per experiment)

Subject: Re: Biohazard Modification: Rylett
From: dwong@robarts.ca
Date: Thu, 08 Oct 2009 12:03:56 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>
CC:

max
C. difficile 2ug
Botulinum neurotoxin
0.1 mg

Hi Jennifer,
Sorry for my late:
1. We will have max of one order only.
2. The Toxins came with powder form and will be dissolved in water or PBS buffer and do it in the fumehood.
Thanks,

Daisy

Jennifer Stanley <jstanle2@uwo.ca> wrote ..

> Hi Daisy:
>
> Just a few last questions (I promise)!
>
> 1. Please confirm that you will only have a maximum of one order of C. difficile (2 ug) or Botulium neurotoxin C (0.1 mg).
> 2. Are the toxins purchased in powder or liquid form? Please explain how you will dilute it (if needed). ie if you are buying a powder, will you dilute the toxin the fumehood, etc. Note any personal protective equipment you will wear.
>
> Thanks
> jennifer
>
> Ron Noseworthy wrote:
>> FYI
>>
>> -----Original Message-----
>> From: dwong@robarts.ca [mailto:dwong@robarts.ca]
>> Sent: September 23, 2009 3:37 PM
>> To: Ron Noseworthy
>> Subject: RE: Biohazard Modification
>>
>> Hi Ron,
>>
>> This is Stef information: she use 12ml of 100ng.ml per experiment.
>> Thanks,
>> Daisy
>>
>> "Ron Noseworthy" <rnoseworthy@robarts.ca> wrote ..
>>
>>> Hi Daisy,
>>>
>>> Thanks, the only other thing we need to know (I mentioned this morning
>>>
>>> at the inspection) is what is the LD50 for both toxins and what species is the LD50 for?
>>>
>>> Thanks

> Ron
>
> -----Original Message-----
> From: dwong@robarts.ca [mailto:dwong@robarts.ca]
> Sent: September 23, 2009 2:22 PM
> To: Ron Noseworthy
> Subject: RE: Biohazard Modification
>
> Hi Ron,
>
> I have try my best to get the information from them:
> 1. a. Clostridium difficile toxin B - 2ug (one order)
> b. Botulinum neurotoxin C - 0.1mg (one order) 2. It will be stored in a locked box in 4oC for Clostridium and -20oC for Botulinum
>
> fridge.
>
> 3. a. 1ug/ml each time.
> b. I am still waiting for Stef's reply.
> 4. For the inventory will do it as radioactive subtance (do record everytime how much is used..) Once I recevied information from Stef
>
> I
>
> will e-mail you.
>
> Thank you very much
> Daisy
>
> "Ron Noseworthy" <rnoseworthy@robarts.ca> wrote ..
>
>> Hi Daisy,
>>
>> Can you respond to these questions if you do get the toxin.
>> 1) For each toxin how much will you have on hand.
>>
>> 2) How much will you store and where will it be stored.
>>
>> 3) How much will be used each time.
>>
>> Also, you will need to keep an inventory of each toxin.
>>
>> Thanks
>>
>> Ron
>>
>> -----Original Message-----
>> From: dwong@robarts.ca [mailto:dwong@robarts.ca]
>> Sent: September 21, 2009 9:39 AM
>> To: Ron Noseworthy
>> Subject: Re: Biohazard Modification
>>
>> Hi Ron,
>>
>> We haven't brought any toxins so far because it take long to process
>>
>> so that why Jane not arrowed them to order. On the other, I have already done all the paperwork, I think just keep in the licence.

Subject: FW: Biohazard Modification
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Wed, 23 Sep 2009 16:29:19 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

questions sent Oct 5/09

FYI

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To: Ron Noseworthy
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3) How much will be used each time.

Also, you will need to keep an inventory of each toxin.

Thanks

Ron

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Regards,
Daisy

"Ron Noseworthy" <rnoseworthy@robarts.ca> wrote ..

Hi Daisy,

They have a few more questions regarding the toxins. Please rewrite and answer the following questions.

1) For each toxin how much will you have on hand.

2) How much will you store and where will it be stored.

3) How much will be used each time.

Also, you will need to keep an inventory of each toxin.

Thanks and let me know if you have any questions.

Ron
Ron Noseworthy MCIC CRSP
Manager, Occupational Health and Safety Robarts Research Institute

UWO, Schulich School of Medicine and Dentistry 100 Perth Drive,

P.O. Box 5015 London, Ontario N6A 5K8
Phone: 519-663-5777 ext. 24125
Fax: 519-931-5267

SIGMA-ALDRICH

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.

SIGMA-ALDRICH

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 4.

*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 ⊗ 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 ⊗ 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 1.0 to 9.0 please indicate the highest HC 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: _____ Expiry Date (3 years from Approval): _____

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	**Price:**	**$256.00**
Designations:	293 [HEK-293]	**Depositors:**	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**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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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:	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

PHOTO

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