Modification Form for Permit BIO-RRI-0043

Permit Holder: Michael Poulter

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

Vladimir Zhurov

~~Douglas Salgado~~

~~Chris Drummond Main~~

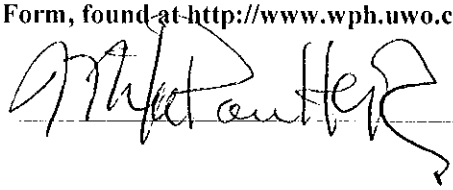
Additional Personnel
(Please list additional personnel here)

Fiona Simpson
Michelle Everest

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha	
Approved Cells	rodent (primary), human (established), E-18 embryo, HEK-293, IMR-32	rat B35 neuroblastoma
Approved Use of Human Source Material	Human brain (unpreserved)	
Approved GMO	Adeno E1A, E. coli DH5 alpha	
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.

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

Signature of Permit Holder: 

Classification: 2

Date of Last Biohazardous Agents Registry Form: Oct 19, 2007

Date of Last Modification (if applicable):

BioSafety Officer(s):

Chair, Biohazards Subcommittee:

Modification for Permit BIO-RRI-0043

Rat B35 Neuroblastoma Cells

B35 cells will be cultured for use in transient transfection experiments and treated with drugs such as valproic acid, trichostatin A and carbemazipine.

Cell Biology

ATCC® Number:	**CRL-2754™**	Order this Item	Price:	**$338.00**

Designations:	B35
Depositors:	P Maness
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	neuronal 
Source:	**Organ:** central nervous system (CNS) **Strain:** BD1X **Disease:** neuroblastoma **Cell Type:** neuronal neuroblast; nitrosoethylurea (NEU) induced
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.
Age:	4 to 10 months
Comments:	Rats were inoculated with N-nitrosoethylurea (NEU) 15 days after conception. Tumors found in the central nervous system (CNS) 4 to 10 months after birth were excised, minced, adapted to culture and cloned [PubMed: 4151463]. B35 cells can be stimulated to differentiate in the presence of dibutyryl cyclic AMP (cAMP) or by serum deprivation. They are easily transfected with plasmid DNA. The cells retain glutamic acid decarboxylase (GAD) and choline acetyltransferase activities; express gamma aminobutyric acid (GABA). The cells are negative for S100 (S-100) protein [PubMed: 4151463]. The cells are positive for neuron specific enolase [PubMed: 6722796].The cells also may be used to study the metabolism and physiology of nervous tissue and the pathology of nervous disorders. A culture submitted to the ATCC in October 2002 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with BM Cycline. The cells were assayed for mycoplasma, by the Hoechst stain, PCR and the standard culture test, after a six-week period following treatment. All tests were negative.

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Protocol:** Subculture before confluency
Subculturing:	1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:5 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	61205: Schubert D, et al. Clonal cell lines from the rat central nervous system. Nature 249: 224-227, 1974. PubMed: 4151463 61314: Vinores SA, et al. Immunoradiometric and immunohistochemical demonstration of neuron-specific enolase in experimental rat gliomas. Cancer Res. 44: 2595-2599, 1984. PubMed: 6722796 88865: Otey CA, et al. B35 neuroblastoma cells: an easily transfected, cultured cell model of central nervous system neurons. Methods Cell Biol. : 287-304, 2003. PubMed: 12884695

Return to Top

BIO-RRI-0043

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

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

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

PRINCIPAL INVESTIGATOR Michael O Poulter
SIGNATURE M.O. Poulter
DEPARTMENT Physiology
ADDRESS Robarts Research.
PHONE NUMBER 663-3450
EMAIL mpoulter@robarts.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 3-04
*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
GABA$_A$ receptor Structure and Function.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NSERC

Names of all personnel working under Principal Investigators supervision in this location:
i) Chris Drummond Main.
ii) Douglas Salgado
iii) Vladimir Zhorov
iv)
v)

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

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
E. coli DH5α	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required. 1 2 3

1.4 Source of microrganism(s) or biological agent(s)? _____

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	☐ Yes ☐ No	
Rodent	☒ Yes ☐ No	E18-embryo
Non-human primate	☐ Yes ☐ No	
Other (specify)		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	☒ Yes ☐ No	HEK-293, IMR-32	ATCC
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☑YES ☐NO
If no, please proceed to Section 4.0

3.2 Indicate if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human organs (unpreserved) ☑ YES ☐ NO If YES, Specify brain biopsy
- Human tissues (unpreserved) ☐ YES ☐ NO If YES, Specify _____

3.3 Is human source known to be infected with and infectious agent ☐ YES ☑ NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 (2) 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 ☑ NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

- HIV ☐ YES ☐ NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐ YES ☐ NO
if YES specify_____
- Other human or animal pathogen and or their toxins ☐ YES ☐ NO
if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☐ YES ☐ NO If YES specify_____
- Known oncogenes ☐ YES ☐ NO If YES specify_____
- Adeno E1A ☑ YES ☐ NO

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES ☐ NO
If YES name virus_____.

4.5 List specific vector(s) to be used: E. Coli DH5α

4.6 Will virus be replication defective ☐ YES ☐ NO

4.7 Will virus be infectious to humans or animals ☐ YES ☐ NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? ☐ 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? ☐ YES ☐ NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☐ 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 If using murine cell lines, have they been tested for murine pathogens? ☐ YES ☐ 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 bodily fluids including blood be used:

- Pound source dogs ☐ YES ☑ NO
- Pound source cats ☐ YES ☑ NO
- Sheep or goats ☐ YES ☑ NO
- Non- Human Primates ☐ YES ☑ NO If YES specify species _____
- Wild caught animals ☐ YES ☑ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin_____

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

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

9.0 Import Requirements

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

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

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

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

10.0 Training Requirements for Personnel named on Form

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

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

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 _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 (2) 3

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

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

12.0 Approvals

UWO Biohazard Subcommittee

Signature G Kidder Date 19 Oct. '07

Safety Officer for Institution where experiments will take place

Signature J L Hawley Date Oct 17/07

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

Signature_____ Date_____

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