THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

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

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

PRINCIPAL INVESTIGATOR	Robert Cumming
DEPARTMENT	Department of Biology
ADDRESS	BGS, Room 3078
PHONE NUMBER	519-661-2111, ext 81578
EMERGENCY PHONE NUMBER(S)	519-601-2733
EMAIL	rcummin5@uwo.ca

Location of experimental work to be carried out: Building(s) BGS Room(s) 3082

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): Subcellular analysis of the disulfide proteome in mammalian cells

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Jordan Epstein	jepstei@uwo.ca	June-16-2010
Robert Arseneault	rarsenea@uwo.ca	June-17-2010
Kyle Dailey	kdailey@uwo.ca	Oct-12-2009
Tyler Cann	tcann@uwo.ca	Sept-23-2009

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

My lab uses Level 1 and 2 cultured cells derived from rodents and humans. These cells are used to model various aspects of human disease and to assess biochemical functions associated with disease. These cells are stored in freezing media in my -80 degree freezer and in a liquid nitrogen storage vat in my lab. All plasticware that is used for culturing these cells is autoclaved before disposal. All used media collected is treated with bleach overnight before disposal down the sink.

My lab uses DH5-alpha bacteria for propagating plasmid DNA. These cells are stored in my -80 degree freezer. All culture plates and media are autoclaved before disposal.

Please include a one page research summary or teaching protocol.

Redox Regulation of Proteins Involved in Ageing and Disease

Reactive oxygen species (ROS) are involved in a variety of different cellular processes including cytokine mediated signalling, apoptosis, cell proliferation and ageing. Although ROS have traditionally been perceived as agents that cause non-specific damage to biological macromolecules it is now known that ROS selectively modify a wide spectrum of proteins. ROS mediated oxidation of protein cysteine sulfhydryl groups (Cys-SH) can lead to the formation of covalent disulfide bonds (Cys-S-S-Cys). Although disulfide bond formation in eukaryotes has generally been studied in the context of cell surface or secreted proteins that fold within the endoplasmic reticulum, recent studies suggest that disulfide bond formation occurs within multiple subcellular environments including the cytosol, mitochondria and the nucleus. However, the effect of disulfide bonding on the function, stability and intracellular transport of many redox sensitive proteins is just now being explored. In addition, cells that primarily use glycolysis to meet cellular energy requirements, exhibit decreased ROS production and resistance to apoptosis. Research in my lab focuses on the effect of altered metabolism and associated redox modifications of proteins implicated in several age-related disorders, including Huntington's disease (HD), Alzheimer's disease (AD) and cancer. Current projects include:

1) Identification of redox sensitive proteins in Huntington's disease cell models . Oxidative stress has been implicated in HD, an inherited neurodegenerative disorder characterized by the intracellular accumulation of a polyglutamine containing protein (Huntingtin) and the progressive loss of striatal neurons. Preliminary studies in my lab have shown that overexpression of pathogenic Huntingtin in nerve cell lines promotes oxidative stress and increased disulfide bonding of cytoplasmic, mitochondrial and nuclear proteins. The redox status of these proteins, many of which have antioxidant functions, are currently being characterized in different HD cell models. In addition, several thiol-based antioxidant compounds are being tested for their ability to prevent mutant Huntingtin induced disulfide bond formation and toxicity.

2) Exploring the role of increased glycolytic metabolism in amyloid-beta resistant nerve cells. Accumulation of the amyloid-beta peptide within the brain tissue of AD patients is strongly linked to oxidative stress and neurotoxicity. Nerve cell lines that are resistant to amyloid-beta toxicity display enhanced glycolysis. Projects in my lab examine different glycolytic enzymes and their ability to influence cellular antioxidant defence and resistance to amyloid-beta. Understanding the role that glycolysis plays in amyloid-beta resistant nerve cells can provide novel insight into AD pathogenesis.

3) Evaluating the effect of elevated glycolysis on the disulfide proteome of breast cancer cells. Breast cancer cells exhibit elevated levels of glycolytic enzymes and the increased production of lactic acid compared to non-transformed cells. This altered metabolism, strongly associated with cancer, also has profound effects on the redox

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☑ YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
DH5α bacteria	O Yes ☑ No	O Yes ☑ No	O Yes ☑ No	0.2L	Invitrogen	☑ 1 O 2 O 2+ O 3
E.coli						O 1 O 2 O 2+ O 3
						O 1 O 2 O 2+ O 3
	O No	O No	O No			O 1 O 2 O 2+ O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☑ YES 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:

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:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☑ Yes O No	U87-MG MDA-MB-435	Level 1	ATCC
Rodent	☑ Yes O No	PC12, HT22, B12	Level 1/2	The Salk Institute
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 ☑ 1 ☑ 2 O 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 Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

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

E.coli

done? O YES, complete table below O NO

			Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5 alpha	pcDNA3.3	Invitrogen		PDK1	Altered mitochondrial metabolism

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below ☒ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 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 ☒ YES O NO
- E1A oncogene O YES ☒ 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? N/A O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES ☒ NO

5.3 How will the biological agent be administered? N/A

5.4 Please give the Health Care Facility where the clinical trial will be conducted: N/A

5.5 Has human ethics approval been obtained? O YES, number: N/A O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used mus musculus

6.3 AUS protocol # To Follow

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ ☒ NO

6.5 Will the agent(s) be shed by the animal: O YES ☒ NO, please justify:

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES ☑ No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

7.4 If no live animals are used, please specify the source of the specimens:

_____

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*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

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

9.0 Insects

9.1 Do you use insects? 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 life stage of the insect? _____

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

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

_____

_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

10.0 Plants

10.1 Do you use plants? 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
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ☑ YES, please give country of origin USA O NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ ☑ 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE Robert Cunning

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. O 1 ☑ 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, date of most recent biosafety inspection: Dec 14, 2010
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): B10-UWO-0209

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

_____
_____

See E-mail

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

SIGNATURE Robert Cumming Date: Apr 28, 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

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Cell line(s)

ATCC Advanced Catalog Search » **Product Details**

Product Description

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

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

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Cell Biology

ATCC® Number:	HTB-14™ Order this Item	**Price:**	$279.00
Designations:	U-87 MG		
Depositors:	J Ponten		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	**Organ:** brain **Tumor Stage:** classified as grade IV as of 2007 **Disease:** glioblastoma; astrocytoma		
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.		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Tumorigenic:	Yes		
Antigen Expression:	Blood Type A, Rh+		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,11 D13S317: 8,11 D7S820: 8,9 D5S818: 11,12 D16S539: 12 vWA: 15,17 THO1: 9.3 TPOX: 8		
Cytogenetic Analysis:	This is a hypodiploid human cell line with the modal chromosome number of 44 occurring in 48% of cells. The rate of higher ploidy was 5.9%., Twelve markers were common to all cells, including der(1)t(1;3)(p22;q21), der(16)t(1;16)(p22;p12), del(9)(p13) and nine others. The marker der(1) had two copies in most cells. There was only one copy of normal X. N1, N6 and N9 were not found.		

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

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

Age: 44 years

Gender: female

Ethnicity: Caucasian

Comments: This is one of a number of cell lines derived from malignant gliomas (see also ATCC HTB-15 and ATCC HTB-16) by J. Ponten and associates from 1966 to 1969.
Mycoplasma contamination was eliminated in September 1975.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: 5% CO2 in air recommended
Temperature: 37.0°C

Subculturing: **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:5 is recommended
Medium Renewal: 2 to 3 times per week
Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
- 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 37C to facilitate dispersal.
- Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting
- Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at 37C.

Preservation: Culture medium, 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020

References:
22159: Beckman G, et al. G-6-PD and PGM phenotypes of 16 continuous human tumor cell lines. Evidence against cross-contamination and contamination by HeLa cells. Hum. Hered. 21: 238-241, 1971. PubMed: 4332744
22536: Fogh J, et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. 58: 209-214, 1977. PubMed: 833871
22539: Fogh J, et al. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59: 221-226, 1977. PubMed: 327080
23094: Olopade OI, et al. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res. 52: 2523-2529, 1992. PubMed: 1568221
23128: Ponten J, Macintyre EH. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. 74: 465-486, 1968. PubMed: 4313504
32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: 8855306
16173472: Clark MJ, et al. U87MG decoded: The genomic sequence of a cytogenetically aberrant human cancer cell line. PLoS Genetics 6 (1) : e1000832, 2010.

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ATCC Advanced Catalog Search » **Product Details**

Product Description

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

Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, 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-2914™	Order this Item	MDA-MB-435	**Price:** $379.00
Designations:	M4A4	MDA-MB-435		
Depositors:	D Tarin			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	*Homo sapiens* (human)			
Morphology:	epithelial			

Related Links

- NCBI Entrez Search
- Cell Micrograph
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Source:	**Organ:** See note in the comments below **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1992
Receptors:	epidermal growth factor (EGF), expressed
Oncogene:	c-myc; Ras; p53
Antigen Expression:	CD44; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 11,12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Age:	31
Gender:	female
Ethnicity:	Caucasian

Comments:

The parental cell lines CRL-2914 (M4A4) and CRL-2918 (NM2C5) were derived from the human breast cancer cell line, MDA-MB-435. [PubMed: 7683291]. M4A4 is highly metastatic in immuno-deprived mice, while NM2C5 is weakly or virtually non-metastatic. These well characterized, tumorigenic human isogenic cell lines have dramatically opposite metastatic phenotypes and are ideal for metastatic studies.M4A4 LM3-4 CL 16 GFP (CRL-2917) cell line was derived from a third generation lung metastasis after inoculation of M4A4 GFP cells in a nude mouse mammary gland. The M4A4 GFP (CRL-2915) was developed by the transduction of the GFP gene into M4A4 (CRL-2914) cell line. One of the isolated cell lines, M4A4 LM3-2 GFP (CRL-2916) was derived from a second lung metastasis.
Note: Recent studies have generated questions about the origin of the parent cell line, MDA-MB-435. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCC has responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line. [16173089] [16173090] [16173091] [16173092] [16173093]

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%.
Atmosphere: 5% CO2 in air recommended
Temperature: 37.0°C

Subculturing:

Protocol: To avoid phenotypic drift it is recommended to make frozen aliquots of the cells and use each aliquot for only 10 passages.

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

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 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 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 1 X 10(3) to 3 X 10(3) viable cells/sq. cm is recommended.
5. Incubate cultures at 37C. We recommend that you maintain cultures at a cell concentration between 8 X 10(4) and 1 X 10(5) cells/sq. cm.
   Subcultivation Ratio: A subcultivation ratio of 1:10 to 1:20 is recommended
   Medium Renewal: 2 to 3 times a week

Preservation:

Freeze medium: complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Doubling Time:

about 30 hours

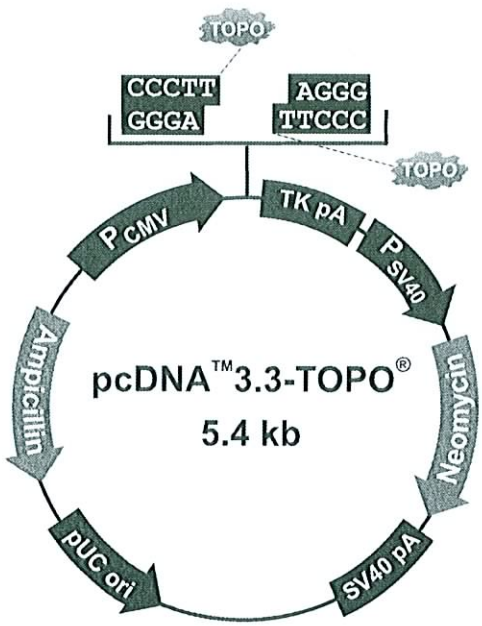
Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
phosphate-buffered saline:ATCC 30-2200
Cell culture tested DMSO:ATCC 4-X
Erythrosin B vital stain solution:ATCC 30-2404
Trypan Blue vital stain solution:ATCC 30-2402
derived from same cell line:ATCC CRL-2915
derived from same cell line:ATCC CRL-2918
derived from same cell line:ATCC CRL-2919
derived from same cell line:ATCC CRL-2916
derived from same cell line:ATCC CRL-2917

Map and Features of pcDNA™3.3-TOPO®

Map

The map below shows the elements of the pcDNA™3.3-TOPO® vector. **The complete sequence is available for downloading from www.invitrogen.com or by contacting Technical Support (page 26).**



Comments for pcDNA™3.3-TOPO® 5407 nucleotides

CMV promoter:	47-726
CMV forward primer binding site:	584-604
TOPO® cloning site:	741
TK pA reverse primer binding site:	787-805
TK polyadenylation signal:	780-1051
f1 replication origin:	1087-1515
SV40 early promoter:	1520-1889
Neomycin Resistance gene:	1925-2719
SV40 polyadenylation signal:	2895-3025
pUC origin (c):	3408-4081
Ampicillin (*bla*) resistance gene (c):	4226-5086
bla promoter (c):	5087-5185

(c) = complementary strand

Plasmid(s)

Continued on next page

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

MSDS'

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ-PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Subject: Re: Biological Agents Registry Form: Cumming lab
From: Robert Cumming <rcummin5@uwo.ca>
Date: Thu, 05 May 2011 10:37:11 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Please find attached the response protocol for accidental biological agent exposure in my lab.

Regards,

Rob

E-mail

Robert Cumming, Ph.D.
Assistant Professor
Department of Biology,
Biological & Geological Sciences Building, Room 3078
University of Western Ontario,
London, Ontario, Canada N6A 5B7
email: rcummin5@uwo.ca
Phone: 519-661-2111 ext. 81578

----- Original Message -----
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tuesday, May 3, 2011 4:13 pm
Subject: Biological Agents Registry Form: Cumming lab
To: Robert Cumming <rcummin5@uwo.ca>

> Dr. Cumming -
>
> Thanks for your recent submission.
>
> I noticed that the following question was not answered; please respond
> by e-mail:
> Question 14.2
> Please outline what will be done if there is an exposure to the
> biological agents listed, such as a needlestick injury or an
> accidentalsplash.
> Regards
> Jennifer

--

Needlestick-splash response.doc	**Content-Type:** application/msword **Content-Encoding:** base64

Accidental biological agent exposure response

All needle stick injuries will be reported to UWO using the form found at:
http://www.uwo.ca/humanresources/docandform/forms/ohs/aiir.pdf
- Completed and signed report will be submitted by supervisor to OHS within 24 hours of the incident

Procedures to be followed in the event of a needlestick/sharps injury

- Encourage bleeding of the injury site
- Wash site thoroughly with soap and water
- Cover area with sterile dressing if necessary
- seek a medical assessment if necessary

For accidental splashes on the skin, the site will be washed thoroughly with soap and water. Protective eyewear will be worn at all times in the lab. In the event of accidental eye exposure to a biological agent, the student/employee will make use of the eye wash station in my lab to thoroughly flush eyes with water.