Modification Form for Permit BIO-RRI-0026

Permit Holder: Caroline Schild Poulter

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

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

Louisa Salemi

~~Robert Dale~~

Victoria Fell

Elnaz Atabakhsh

Additional Personnel
(Please list additional personnel here)

Keshini Devakandan
Daniel ~~Woelf~~ Worndl
Samantha Hershenfeld
Xu Wang

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	E.coli (DH5 alpha, HB101), S. cerevisiae	
Approved Primary and Established Cells	[Established] (Human): HeLa, HEK293, HCT 116, MCF-7, Phoenix (HEK293-derivative). (Rodent): mouse embryonic fibroblast	(Human) IHR90 HCF-10A IHR-32
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] - p.Super.Neo. [Vector]: pMSCV	
Approved Use of Animals		
Approved Biological Toxin(s)		Cholera Toxin
Approved Gene Therapy		

Approved Plants and Insects

As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder:

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

Date of Last Biohazardous Agents Registry Form: Nov 24, 2009

Date of Last Modification (if applicable): Feb 14, 2012

BioSafety Officer(s)*: Feb. 27, 2012

For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.

Chair, Biohazards Subcommittee: **Date:**

Additional cell lines are primary cells and will be used to test

Additional cell lines being added to permit are primary cells that will be used to repeat experiments previously performed in cancer cell lines. Cells will be stored in liquid Nitrogen when not in use, and will be disposed of by bleaching and biohazardous waste disposal.

The Cholera Toxin will be used as a media supplement for MCF10A cells, which require the toxin in order to grow. We will purchase 1mg of the toxin for our uses. The LD50 of the toxin in mice is 250ug/kg

Cell Biology

ATCC® Number: **CCL-186™** Order this Item Price: **$279.00**

Designations:	IMR-90
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	fibroblast
Source:	**Organ:** lung **Disease:** normal **Cell Type:** fibroblast
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
Virus Susceptibility:	Human poliovirus 1 Human poliovirus 2 Varicella-Zoster Herpes simplex virus 1 Herpes simplex virus 2 Human poliovirus 3 Vaccinia virus Human herpesvirus 5 Vesicular stomatitis virus
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 11,13 D16S539: 10,13 D5S818: 12,13 D7S820: 9,12 THO1: 8,9.3 TPOX: 8,9 vWA: 16,19
Cytogenetic Analysis:	normal human female; diploid; stable
Isoenzymes:	G6PD, B
Age:	16 weeks gestation
Gender:	female
Ethnicity:	Caucasian

Related Links

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Related Cell Culture Products

Login Required

Product Information Sheet

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management: basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

Comments:	The human diploid fibroblast strain IMR-90 was derived by W.W. Nichols and associates from the lungs of a 16-week female fetus. The division potential, viral susceptibilities and other properties have been thoroughly studied such that the line may be considered as an alternate for WI-38 and other standard human lung cell strains. The cells have been reported to be capable of attaining 58 population doublings before the onset of senescence.
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:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that 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 1:2 to 1:8 is recommended **Medium Renewal:** Every 3 to 4 days **Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	

22381: Nichols WW, et al. Characterization of a new human diploid cell strain, IMR-90. Science 196: 60-63, 1977. PubMed: 841339
32932: Dolganov GM, et al. Human Rad50 is physically associated with human Mre11: identification of a conserved multiprotein complex implicated in recombinational DNA repair. Mol. Cell. Biol. 16: 4832-4841, 1996. PubMed: 8756642
33041: Ostlund RE Jr., et al. A sterospecific myo-inositol/D-chiro-inositol transporter in HepG2 liver cells. J. Biol. Chem. 271: 10073-10078, 1996. PubMed: 8626564

Return to Top

Cell Biology

ATCC® Number:	**CCL-127™**	Order this Item	Price:	**$279.00**

Designations:	IMR-32
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	fibroblast; neuroblast 
Source:	**Organ:** brain **Disease:** neuroblastoma **derived from metastatic site:** abdominal mass
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:** April, 1967
Applications:	transfection host
Virus Resistance:	echovirus 11
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9 D16S539: 8 D5S818: 11,12 D7S820: 9,10 THO1: 7,9.3 TPOX: 11 vWA: 15
Cytogenetic Analysis:	Stable male karyotype with stemline number of 49. Two large marker chromosomes with submedian centromeres. A deletion in one number 1 chromosome: One number 16 chromosome missing; two extra chromosomes in C group. Sublines with 50 and 48 chromosomes differ from those with 49 chromosomes by having an extra or missing C group chromosome respectively.
Isoenzymes:	G6PD, B
Age:	13 months
Gender:	male
Ethnicity:	Caucasian

Related Links

NCBI Entrez Search

Cell Micrograph

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Frequently Asked Questions

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Comments:	The IMR-32 cell line was established by W.W. Nichols, J. Lee and S. Dwight in April, 1967 from an abdominal mass occurring in a 13-month-old Caucasian male. [22190] The tumor was diagnosed as a neuroblastoma with rare areas of organoid differentiation. Two cell types are present. Predominant is a small neuroblast-like cell. The other is a large hyaline fibroblast. The cell line was submitted to the American Type Culture Collection in the 36th passage. It has been demonstrated that the cells can be propagated successfully beyond the 80th serial subculture. CCL-127 cells may pile up and grow in patches. (Please see the photos of CCL-127 on the ATCC website at www.atcc.org). CCL-127 cells may not become 100% confluent.
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%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.Maintain cultures at a cell concentration between 4 X 10 exp4 and 4 X 10 exp5 cells/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Doubling Time:	approximately 20 hrs.
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22190: Tumilowicz JJ, et al. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res. 30: 2110-2118, 1970. PubMed: 5459762 32287: Rostomily RC, et al. Expression of neurogenic basic helix-loop-helix genes in primitive neuroectodermal tumors. Cancer Res. 57: 3526-3531, 1997. PubMed: 9270024 32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

Return to Top

Cell Biology

ATCC® Number:	**CRL-10317™** Order this Item	Price:	**$279.00**

Designations:	MCF 10A
Depositors:	Michigan Cancer Foundation
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** fibrocystic disease **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:** August 22, 1984
Applications:	transfection host
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 8,9 D16S539: 11,12 D5S818: 10,13 D7S820: 10,11 THO1: 8,9.3 TPOX: 9,11 vWA: 15,17
Isoenzymes:	AK-1, 1 [23084] ES-D, 1 [23084] G6PD, B [23084] GLO-I, 1-2 [23084] PGM1, 1-2 [23084] PGM3, 1 [23084]
Age:	36 years
Gender:	female
Ethnicity:	Caucasian

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References:

21968: Soule H, McGrath CM. Immortal human mammary epithelial cell lines. US Patent 5,026,637 dated Jun 25 1991
22025: Pauley RJ, et al. Immortal human mammary epithelial cell sublines. US Patent 5,206,165 dated Apr 27 1993
22248: Soule HD, McGrath CM. A simplified method for passage and long-term growth of human mammary epithelial cells. In Vitro Cell. Dev. Biol. 22: 6-12, 1986. PubMed: 2418007
23084: Soule HD, et al. Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res. 50: 6075-6086, 1990. PubMed: 1975513
23085: Tait L, et al. Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line, MCF-10. Cancer Res. 50: 6087-6094, 1990. PubMed: 1697506

Return to Top

Material Safety Data Sheet

Version 4.3
Revision Date 12/01/2011
Print Date 02/17/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Cholera Toxin Vibrio cholerae

Product Number : C8052
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Bowel

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects — Moderate skin irritant; Moderate eye irritant

GHS Classification

Acute toxicity, Oral (Category 4)
Acute toxicity, Dermal (Category 4)
Skin irritation (Category 3)
Acute aquatic toxicity (Category 3)
Chronic aquatic toxicity (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H302 + H312	Harmful if swallowed or in contact with skin.
H316	Causes mild skin irritation.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

P273	Avoid release to the environment.
P280	Wear protective gloves/ protective clothing.

HMIS Classification

Health hazard: 2
Chronic Health Hazard: *

Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. Causes respiratory tract irritation.
Skin Harmful if absorbed through skin. Causes skin irritation.
Eyes Causes eye irritation.
Ingestion Harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cholera enterotoxin
Cholergen

CAS-No.	EC-No.	Index-No.	Concentration
Tris (hydroxymethyl) aminomethane			
77-86-1	201-064-4	-	>= 5.82 - <= 5.94 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	>= 31.3 - <= 31.9 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	>= 1.83 - <= 1.87 %
Sodium chloride			
7647-14-5	231-598-3	-	>= 57.6 - <= 58.8 %
Exotoxin, vibrio cholerae			
9012-63-9	-	-	>= 0.5 - <= 2.5 %
Sodium azide			
26628-22-8	247-852-1	011-004-00-7	>= 0.96 - <= 0.98 %

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIREFIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Dimethyl sulfate, Acid chlorides, Halogenated hydrocarbon, Metals, Acids

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas, Sodium oxides
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50

no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Harmful if swallowed.
Skin	Harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Signs and Symptoms of Exposure
Laboratory experiments in animals have shown sodium azide to produce a profound hypotensive effect, demyelination of myelinated nerve fibers in the central nervous system, testicular damage, blindness, attacks of rigidity, and hepatic and cerebral effects.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Harmful to aquatic life with long lasting effects.

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate eye irritant

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Text of H-code(s) and R-phrase(s) mentioned in Section 3

Further information

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 Co., 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.

Cholera Toxin

01-511 100 µg

Cholera toxin, a main enterotoxin, interacts with G proteins and increases cyclic AMP in the intestinal lining to open ion channels. As ions flow into the intestinal lumen (lining), body fluids (mostly water) flows out of the body due to osmosis leading to massive diarrhea as the fluid is expelled from the body. Cholera toxin is a complex consisting of one molecule of A subunit (27.2 kD) and 5 molecules of B subunits (11.6 kD). It adsorbs to GM1 ganglioside on the surface of target cells by the B subunit and penetrates into cells where A subunit is dissociated and processed into A1, which constitutively activates adenyl cyclase activity of α subunit of Gs (a kind of GTP-binding protein).

This toxin was highly purified from growth medium of *Vibrio cholerae,* 569B strain.

Applications

1) Detection of GTP-binding protein Gsα
2) Detection of a low molecular weight GTP-binding protein, ARF
3) Manipulation of culture cells to increase cellular concentration of cyclic AMP

Specification

Activity test: Addition of cholera toxin at ~ 1 ng/ml to the culture medium changed more than 50% of Vero cells into spindle shape.

Purity: More than 90% purity (see below; SDS-PAGE)

Form: 400 ug/ml in 50 mM Tris-HCl (pH7.5) 0.2 M NaCl 1mM Na_2EDTA, 10% Glycerol

Storage: -70℃

Data Link Swiss-Prot Cholera toxin

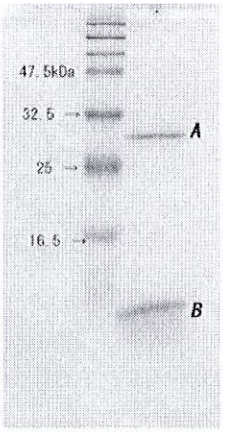


Fig.1 SDS-PAGE of Cholera toxin. A and B indicate the subunit A and B, respectively.

References

1. Hirst TR and D'Souza JM In *The Comprehensive Sourcebook of Bacterial Protein Toxins* Alouf J and Popoff M edt. 3rd edn. p. 270-290 Academic Press (2006)
2. Finkelstein RA and LoSpalluto JJ "Pathogenesis of experimental cholera. Preparation and isolation of choleragen and choleragenoid." *J.Exp Med* **130**: 185-202 (1969) PMID: 4978880
3. Iijima Y and Honda T "Enterotoxin of Vibrio Cholerae." In *Recent Advances in Marine Biotechnology* Fingerman M and Nagabhushanam R edt. Science Pub. Inc. **7**: 41 (2002)

＊This product is for research use only, not for human application.

＊MSDS (Material Safety Data Sheet) is in the next page.

Related products: # 01-521 Cholera toxin subunit A # 01-525 Cholera toxin subunit B

BioAcademia,Inc. Tel. 81-72-643-4660 Fax.81-72-643-4701 info@bioacademia.co.jp http://www.bioacademia.co.jp/en/

Material Safety Data Sheet
Cholera Toxin

Harzardous Ingradient

On a weight basis, cholera toxin constitutes >95% of the total protein (0.4~1.0 mg/ml, depending on lot).

Health Hazard Data

The LD_{50} in mice is 250 μg/kg when injected intravenously. It is believed that human is more susceptible than mouse.

Emergency Procedure

If the toxin is accidentally swallowed, drink large amounts of water or hypotonic drinks, and ask a physician for emergency treatments of cholera diarrhea.

If skin pricking occurs accidentally, bleed and perform vigorous flushing of the area with large amounts of water. If injection occurs, seek a physician' s advice immediately. Hyperimmune globulin, which is not commercially available, is the only antidote.

Handling

It should be handled carefully by persons with expertise in knowledge and techniques for the safe handling of Cholera toxin. Avoid mouth pipetting. Wear protective gloves when handling the toxin. Avoid contact with open wounds. Wash thoroughly any area of the body that makes contact with the toxin. It is recommended that persons who handle the toxin are immunized by diphtheria vaccine.

Inactivation

The toxin can be inactivated by boiling for 30 min.

Toxins of Biological Origin

Biological toxins are produced by certain bacteria, fungi, protozoa, plants, reptiles, amphibians, fish, echinoderma (spiny urchins and starfish), mollusks, and insects.

The EH&S Biosafety Office regulates the **possession, use, and transfer of unfractionated mixtures and purified preparations of biological toxins with a mammalian LD_{50} of ≤ 100 ug/kg body weight, as well as the organisms, both natural and recombinant, which produce these biological toxins.** These are called "Acute Toxins". Registration forms can be found at http://www.ehs.ufl.edu/Bio/default.asp

The following table from the UF EH&S Biological Safety Manual lists LD_{50} values for some biological toxins. Toxins not on this list may still require registration. For more information, please contact the Biosafety Office at 392-1591.

Toxin	LD50 (ug/kg)*
Abrin	0.7
Aerolysin	7.0
Botulinin toxin A	0.0012
Botulinin toxin B	0.0012
Botulinin toxin C1	0.0011
Botulinin toxin C2	0.0012
Botulinin toxin D	0.0004
Botulinin toxin E	0.0011
Botulinin toxin F	0.0025
b-bungarotoxin	14.0
Caeruleotoxin	53
Cereolysin	40-80
Cholera toxin	250
Clostridium difficile enterotoxin A	0.5
Clostridium difficile cytotoxin B	220
Clostridium perfringens lecithinase	3
Clostridium perfringens kappa toxin	1500
Clostridium perfringens perfringolysin O	13-16
Clostridium perfringens enterotoxin	81
Clostridium perfringens beta toxin	0.4
Clostridium perfringens delta toxin	5
Clostridium perfringens epsilon toxin	0.1
Conotoxin	12-30
Crotoxin	82
Diphtheria toxin	0.1
Listeriolysin	3-12
Leucocidin	50
Modeccin	1-10
Nematocyst toxins	33-70
Notexin	25
Pertussis toxin	15

Pneumolysin	1.5
Pseudomonas aeruginosa toxin A	3
Ricin	2.7
Saxitoxin	8
Shiga toxin	20
Shigella dysenteriae neurotoxin	1.3
Streptolysin O	8
Staphylococcus enterotoxin B	25
Staphylococcus enterotoxin F	2-10
Streptolysin S	25
Taipoxin	2
Tetanus toxin	0.001
Tetrodotoxin	8
Viscumin	2.4-80
Volkensin	1.4
Yersinia pestis murine toxin	10

*Please note that the LD50 values are from a number of sources (see below). For specifics on route of application (i.v., i.p., s.c.), animal used, and variations on the listed toxins, please go to the references listed below.

Reference:
1. Gill, D. Michael; 1982; Bacterial toxins: a table of lethal amounts; Microbiological Reviews; 46: 86-94
2. Stirpe, F.; Luigi Barbieri; Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; Biotechnology; 10: 405-412
3. Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the RTECS. 1997. Doris V. Sweet, ed., U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; Cincinnati, Ohio

More Examples of Biological Toxins Which May Require Registration

Aflatoxins	Leiurotoxins
Amanitin	Lipid A - all types
Amphibian venoms	Lipopolysaccharides from all species
Anatoxin A	Maitotoxin
Anthrax toxin	Medamine
Aspergillus sp toxins	Microcystins
Bacillus sp. toxins - all	Mojave toxin
Bordetella sp. toxins	Mycotoxins - all
Botulinum toxins - all	Myotoxins
Brevetoxins	Neurotoxins - all
Bungarotoxins	Notexin
Cardiotoxin	Nodularin
Charybdotoxin	Ochratoxin
Cholera toxins - all	Palytoxin
Ciguatera toxin	Paradoxin
Clostridia species toxins - all	Pertussis toxins - all
Cluepeotoxins	Phalloidin
Cobra venous and all derived toxins	Psilocybine
Cobratoxin	Pseudomonas sp. toxins
Conotoxins - all	Reptile venoms - all
Crotamine	Resiniferatoxin
Dendrodotoxins	Ricin toxins - all
Dinoflagellate neurotoxins	Sapintoxin

Diphtheria toxins	Sarafotoxin
Domoic acid	Saxitoxin
DTX-1 (Dinophysistoxin-1)	Short Neurotoxins
Echinoderm venoms - all	Snake venoms - all
Endotoxins - all	Stable toxins
Enterobacteriaciae toxins - all	Staphylococcus sp. toxins
Enterotoxins - all	Streptonigrin
Escherichia coli toxins - all	Taipoxin
Exotoxin A	Tetanus toxins - all
Fish venoms - all	Tetrodotoxins - all
Fusarium sp. toxins	Textilotoxin
Gliotoxin	Thymeleatoxin
Joco Spider Toxin JSTX-3	Tinyatoxin
Lappaconitines	Toxin II - all types

Toxins Classified as Select Agents

Some biological toxins are classified by the Federal Government as Select Agents (http://www.selectagents.gov/) due to their potential to pose a severe threat to public health and safety. Possession, use, and transfer of these toxins is highly regulated. A complete list can be found at http://www.selectagents.gov/

In small quantities, some of these toxins are exempt from select agent registration. See the table below. Note however that the **possession, use, or transfer of ANY select agent toxin, IN ANY QUANTITY, must be registered with the EH&S Biosafety Office**. Again, forms can be found at http://www.ehs.ufl.edu/Bio/default.asp. **Do not e-mail or fax select agent registrations** to EH&S, hand-deliver or mail these registrations instead.

Exempt Amounts Select Agent Toxins Permissible Per Principal Investigator

HHS (CDC-listed) Toxins	Amount
Abrin	100 mg
Conotoxin	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Shiga-like ribosome inactivating proteins	100 mg
Tetrodotoxin	100 mg
HHS/USDA Overlap Toxins	**Amount**
Botulinum neurotoxins	0.5 mg
Staphylococcal enterotoxins	5.0 mg
Clostridium perfringens epsilon toxin	100 mg
Shigatoxin	100 mg
T-2 toxin	1000 mg

Working with and Disposing of Biological Toxins

Because they can be extremely hazardous, even in minute quantities, biological toxins require strict

safeguards against their inhalation, absorption through skin or mucous membranes (typically due to a splash), ingestion, or percutaneous injury. Information on the safe use of biological toxins can be found at Safety and Health Considerations For Conducting Work With Biological Toxins (.pdf article) and http://www.cdc.gov/od/ohs/biosfty/bmbl4/b4ai.htm

Key points of the guidelines are:

1. Written safety **protocols** to cover the use of the specific toxin(s) in use
2. **Security measures** in place to protect against unauthorized access to toxin(s)
3. **Inventory control** system in place; all entries in a hardbound book, in ink
4. Written **plan for** toxin-related **emergencies** (spill, exposure, etc) posted
5. **BSL-2 or BSL-3** containment and practices in use

Specific inactivation and disposal requirements are in place for acute biological toxins. Some toxins are quite resistant to conventional methods of inactivation. **These agents cannot be simply placed in the biomedical waste or picked up by EH&S Hazardous Waste Services.**

This document is a standard operating procedures (SOP) template for laboratories using acute biological toxins:
UF Toxin SOPs template

Adapted from the University of Pennsylvania EH&S website:
Toxins may be destroyed by several methods as shown in the table below. Some toxins are inactivated by autoclaving for one hour at 121°C. Others are inactivated by exposure to sodium hypochlorite and/or sodium hydroxide.

A. Chemical destruction of toxins:

When using sodium hypochlorite and / or sodium hydroxide to destroy toxin, the procedure(s) must be performed in a laboratory fume hood or a biological safety cabinet. At a minimum, personal protective equipment for all procedures should include:
Long sleeved protective clothing (lab coat, gown)
Gloves and eye protection

1. If the toxin is classified as a select agent, even in exempt amounts, notify EH&S prior to destruction of the agent.
2. Work in a fume hood or biosafety cabinet with the sash at the lowest reasonable sash height for safe and effective work.
3. Place plastic backed absorbent paper (bench diaper) on the work surface of the fume hood or biosafety cabinet.
4. CAREFULLY put the Select Agent toxin into solution in the primary container. DO NOT USE A GLASS CONTAINER.
5. Place the primary container in a secondary container, such as a beaker or rack.
6. Slowly dispense an equal volume of the concentrations of sodium hypochlorite and/or sodium hydroxide designated in table 1 below into the primary container of toxin solution to be destroyed.
7. Do not replace the cap on primary container.
8. Place a "WARNING / DO NOT USE" sign on the hood/cabinet.
9. Allow a minimum 60 minutes exposure time. (See table 1 below for additional exposure time

recommendations.)

10. Document the destruction of the toxin in the laboratory inventory logbook.
11. Secure the cap on the primary container. DOUBLE BAG the material in zip-lock plastic bags and label it "Inactivated/denatured (TOXIN NAME)".
12. Contact EH&S for disposal as hazardous waste.

B. Steam Sterilization (Autoclaving) of Toxins

If acceptable as a method in table 1 below, destroy toxins by autoclaving them using the procedure outlined below:

1. If the toxin is classified as a select agent, even in exempt amounts, notify EH&S prior to destruction of the agent.
2. In a fume hood or biological safety cabinet, loosen the cap of the primary toxin container to allow steam penetration.
3. Place the primary container into a secondary biohazard sharps container.
4. Place the sharps container in a loosely closed biohazard bag.
5. Place the bag in a autoclavable pan.
6. Autoclave at 121° C for 1 hour on liquid cycle (slow exhaust).
7. Document the destruction of the toxin the laboratory inventory logbook.
8. After autoclaving, allow time for materials to cool before handling.
9. Discard the biobag and its containers as biological waste.

DO NOT use steam sterilization for destruction of any of the low molecular weight toxins (i.e. mycotoxins, marine and reptile venoms).

All waste from toxins that is not disposed as infectious waste must be collected by EH&S for disposal as hazardous waste. Call 392-1591.

Toxins classified as select agents, even if in exempt amounts, require that someone from the Biosafety Office observe and document the destruction of these agents. Please call our office (329-1591) to schedule an "observed destruct".

Table 1
Inactivation Procedures for Selected Toxins

Allow at least a 60-minute chemical contact time for complete inactivation of toxin. Any procedure labeled "yes" is an approved procedure for inactivation of the toxin specified.

Toxin	Autoclave (1 hour @ 121° C, liquid exhaust)	2.5% NaOCL + 0.25 N NaOH	1.0% NaOCl	2.5% NaOCl
Abrin (1)(8)	Yes	N/A	N/A	N/A
Botulinum Neurotoxin (1) (7)	Yes	Yes	Yes	Yes
Clostridium perfringens epsilon toxin (2)	Yes	N/A	N/A	N/A
Conotoxin(3)	CALL EH&S			
Diacetoxyscirpenol(5)	No	Yes	No	Yes (3-5%)

Ricin (1)(7)	Yes	Yes	Yes	Yes
Saxitoxin (1)(7)	No	Yes	Yes	Yes
Shigatoxin & Shiga-like ribosome inactivating proteins(4)	Yes	Yes	Yes	Yes
Staphylococcal Enterotoxins (1)(7)	Yes	Yes	Yes	Yes
Tetrodotoxin (1)(7)	No	Yes	Yes	Yes
T-2 Toxin (1)(6)(5)	No	Yes	No	No

1. Wannemacher R.W. 1989. Procedures for Inactivation and Safety Containment of Toxins. Proc. Symposium on Agents of Biological Origin, U.S. Army Research, Dev. and Engineering Center, Aberdeen proving Ground, MD. pp. 115-122
2. Factsheets on Chemical and Biological Warfare, http://www.cbwinfo.com/Biological/Toxins/Cper.html
3. Factsheets on Chemical and Biological Warfare, http://www.cbwinfo.com/Biological/Toxins/Conotox.html
4. Factsheets on Chemical and Biological Warfare, http://www.cbwinfo.com/Biological/Toxins/Verotox.html
5. Factsheets on Chemical and Biological Warfare, http://www.cbwinfo.com/Biological/Toxins/mycotoxins.html
6. For complete inactivation of T-2 mycotoxin extend exposure time for liquid samples, spills, and non-burnable waste in 2.5% sodium hypoclorite and 0.25 N sodium hydroxide to 4 hr. Expose cages/bedding from animals exposed to T-2 mycotoxin to 0.25% sodium hypochlorite and 0.025 N sodium hydroxide for 4 hrs.
7. For inactivation of saxitoxin, tetrodotoxin, ricin, botulinum toxin, or staphylococcal enterotoxins, expose work surfaces, solutions, equipment, animal cages, spills to 10% sodium hypochlorite for 60 minutes.
8. http://www.inchem.org/documents/pims/plant/abruspre.htm

Please don't hesitate to contact the EH&S Biosafety Office with any questions regarding biological toxins.

You can reach us at: 352-392-1591, E-mail: bso@ehs.ufl.edu

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	µg
Proposed Storage Dose:	1000 µg
LD $_{50}$ (species):	250 µg

Calculation:

250 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 12500

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 1250

Comments/Recommendations:

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 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 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	Caroline Schild-Poulter
SIGNATURE	
DEPARTMENT	Robarts Research
ADDRESS	100, Perth Drive
PHONE NUMBER	519-663-5777 ext 24164
EMERGENCY PHONE NUMBER(S)	519-902-6495
EMAIL	cschild-poulter@robarts.ca

Location of experimental work to be carried out: Building(s) ___Robarts_____ Room(s)__3250_

*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:_____NSERC_/ CIHR (applied)_____
GRANT TITLE(S): _____ Molecular determinants of Ku function in non-homologous end-joining and DNA damage signaling pathways _/ Mechanisms of RanBPM-mediated cell death _____

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Elnaz Atabakhsh
Victoria Fell
Robert Dale
Louisa Salemi

*** 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 biological agents? **X** YES O NO
(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 **X** 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 (DH5α, HB101)	X Yes X No 20	**X** Yes O No	O Yes **X** No	2 lt	colleagues	**X** 1 O 2 O 3
S. cerevisiae	X Yes X No 30	**X** Yes O No	O Yes **X** No	2 lt	Clontech	**X** 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

*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? **X** 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 **X** No		Not applicable
Rodent	O Yes **X** No		
Non-human primate	O Yes **X** No		
Other (specify)	O Yes **X** No		

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

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)*	Supplier / Source
Human	**X** Yes O No	HeLa, HEK293, HCT116, MCF-7, Phoenix (HEK293-derivative)	ATCC
Rodent	**X** Yes O No	Established mouse embryonic fibroblasts	Non-commercial
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 **X** 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES **X** 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/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 Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 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? **X** YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli	*pSuper.Neo*	*Oligoengine*	*siRNA*	*Represses selected gene expression*

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

4.3 Will genetic modification(s) involving viral vectors be made? **X** YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Murine stem cell virus	*pMSCV*	*Clontech*	*Ku*	*Re-introduces gene expression in knockout cell line.*

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? **X** YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES **X** 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 O NO

5.3 How will the biological agent 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 **X** 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 in section 4.0 be used in live animals O YES, specify: _____ O NO

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

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

_____

_____

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X 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 detais:_____. O NO

*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 X 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 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***

* 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 **X** 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 NO, please forward the permit to he Biosafety Officer when available.

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 12.0 **X** 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

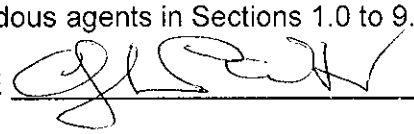
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

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

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

13.2 Has the facility been certified by OHS for this level of containment?

X YES, permit # if on-campus_BIO-RRI-0026_
O NO, please certify
O 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.wph.uwo.ca/

SIGNATURE _____ Date: NOV 3, 2009

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

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

_____
_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 24 NOV. 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: November 23, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: NOV 23/09

Approval Number: BIO-RRI-0026 Expiry Date (3 years from Approval): NOV 23, 2012

Special Conditions of Approval:

Viral vector policy attached (FYI only)

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

NSERC - finded

Title : Molecular determinants of Ku function in non-homologous end-joining and DNA damage signaling pathways

Ku is an abundant, ubiquitous protein for which homologs have been found from bacteria to man. Ku is a DNA binding protein that has proven to be a central player in DNA metabolism. It is involved in telomere maintenance and has been implicated in DNA replication, and transcriptional regulation. The most prominent function of Ku is its essential role in DNA end-joining, which is central to DNA double-stranded break (DSB) repair and V(D)J recombination. In addition to its role in the repair of DNA breaks, Ku also regulates signaling pathways that modulate cellular activities following DNA damage. Ku's biological functions are mediated in part through its interaction with DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a large ser/thr protein kinase for which Ku acts as the DNA-binding subunit. Additionally, Ku interacts with several other factors involved in various nuclear functions.

Ku is a heterodimer of 2 subunits, Ku70 and Ku80, the products of 2 distinct genes that may have one common ancestor gene. Crystal structure analysis has revealed that the heterodimeric complex has an almost symmetrical structure, consistent with the similarities noted in the primary sequence of the two subunits. Both Ku subunits display three structurally similar domains: an amino-terminal alpha/beta domain, a central beta-barrel domain and alpha-helical carboxy-terminal arm. While the central domain is involved in DNA binding, the N- and C-terminal regions engage in protein-protein interactions. Ku has limited enzymatic activity, therefore most of its functions in DNA repair and signaling are though to be imparted by its ability to interact with and modulate regulatory proteins. While several factors have been shown to interact with either subunit, the nature and the outcome of these interactions are still poorly understood.

Here I propose to investigate the contribution of Ku N-terminal domain to Ku's functions in the cellular response to DSBs. The amino terminal domains of Ku70 and Ku80 are highly conserved and share some similarity with von Willebrand factor A domains that are known to mediate protein-protein interactions. **The hypothesis that guides this proposal is that Ku function in DNA damage response and repair pathways is dependent on physical interaction with regulatory factors through discrete protein motifs in these domains.**

We will produce Ku mutants bearing specific point mutations in Ku N-terminal regions that display high level of conservation between species and that are accessible to protein-protein interactions as predicted by the crystal structure. Ku80 and Ku70 mutants, along with the wild-type (Wt) will be expressed in Ku70- or Ku80-deficient mouse embryonic fibroblasts (MEFs) using a retroviral vector strategy. Ku-deficient cells show increased sensitivity to IR leading to a notable increase in cell death in response to IR treatment. The first step will be to assess the ability of these mutants to restore cell survival in response to IR. Mutants that show cell survival defects compared to cells expressing Wt Ku will be selected and further analyzed to identify specific defects in DNA repair. Mutants that confer DNA repair deficiencies will be further investigated to identify potential defects in their interaction with known components and associated factors of the NHEJ repair complex. Cells that express mutants that do not show obvious DNA repair defects will be analyzed to identify alterations in the signaling pathways triggered by DSBs.

In the short term, we expect to identify specific motifs in Ku that regulate its activity in response to DNA damage. In the long-term, these analyses will enhance our understanding of the regulatory function of Ku in the DNA repair and in the regulation of signaling pathways that are activated by double-stranded DNA breaks.

CIHR - Applied

Summary of research proposal

DNA damage is the underlying cause of genomic instability that leads to cell transformation and the development of cancer. In response to DNA damage, eukaryotic cells trigger signaling pathways to induce cell cycle checkpoints, modulate gene transcription and establish DNA repair complexes. The activation of apoptosis is also an essential component of the DNA damage response that prevents cells with genomic alterations from proliferating to a malignant state. Conversely, chromosomal instability favors the inactivation of apoptotic pathways to select for resistant cells which can lead to their proliferation to a malignant state.

RanBPM (Ran-binding protein M) has previously been implicated in various cellular functions including cell signaling, protein stabilization and transcriptional regulation, but its function remains unclear. Our recent investigations have implicated RanBPM in the activation of apoptotic pathways. Specifically, we have found that siRNA-mediated downregulation of RanBPM in Hela cells compromises the induction of apoptosis in response to ionizing radiation (IR). This correlated in a magnitude fold increase survival of RanBPM-depleted cells treated with IR. Conversely, ectopic expression of RanBPM induced cell death through caspase activation. Following IR treatment, endogenous RanBPM was found to translocate from the nucleus to the cytoplasm suggesting that the activation of apoptotic pathways by RanBPM in response to DNA damage may be regulated by nucleocytoplasmic trafficking. Strikingly, RanBPM-deficient cells displayed reduced Bax mitochondrial localization, whereas the overall protein levels of Bcl-2 were substantially up-regulated. These results were corroborated in another cell line, HCT116. Overall, our results reveal a novel function for RanBPM in the regulation of DNA damage-induced apoptotic pathways through the regulation of mitochondrial apoptotic factors.

We hypothesize that RanBPM controls the expression/activity of proteins that are critical regulators of apoptosis. The goal of this project is to elucidate the mechanisms through which RanBPM functions to regulate the activation of apoptotic pathways in response to DNA damage. In **the first objective,** we will address the possibility that RanBPM regulates several factors/pathways that control the activation of apoptosis. First, we propose to determine the contribution of Bcl-2 regulation of expression to the activation of apoptotic pathways by RanBPM in Hela and HCT116 RanBPM-deficient cells. Next, we will investigate other factors/pathways whose regulation is altered by RanBPM downregulation. Finally, we will investigate the nature of the stress signals that trigger RanBPM activation of apoptosis to determine if apoptotic pathways activated by RanBPM are elicited by a wide range of DNA damaging agents, or are restricted to specific types of DNA lesions. In **the second objective**, we propose to identify RanBPM domains that are responsible for its pro-apoptotic function. Analysis of systematic deletions of RanBPM subdomains will identify regions of the RanBPM protein that contribute to the regulation of apoptotic pathways. We will then focus on the identification of nuclear import/export signals to determine how RanBPM subcellular localization affects its ability to activate apoptosis in response to DNA damage. Lastly, we will start investigating the nature of the pathways that trigger its relocalization in response to DNA damage. In **the third objective,** we propose to start investigating the mechanisms through which RanBPM exerts its function. We will initially focus our investigation on the regulation of Bcl-2 by RanBPM to pursue our recent findings which suggest that RanBPM controls Bcl-2 levels at least in part at the transcriptional level. We propose to identify Bcl-2 promoter regions regulated by RanBPM and examine whether RanBPM regulation occurs through a direct effect of RanBPM at the promoter, or through a control of upstream regulatory factors.

We expect that the results we obtain during the term of this grant will provide significant insight into the means and the pathways through which RanBPM induces apoptosis and elucidate the contribution of this factor to the regulation of programmed cell death induced by DNA damage, a fundamental process that contributes to the maintenance of genomic integrity. Also, as most anti-cancer drugs target DNA, the identification of a novel apoptotic pathway activated by DNA damage may also be expected to lead to the development of new strategies to enhance the apoptotic effects of anti-cancer drug therapies.

Policy on Research Utilizing Virus Vector Transduced Cells or Virus Infection of Animals
Version 5
Approved by Biosafety Committee: June, 2009

Research with cells transduced with replication competent or defective viral vectors capable of infecting human or animal cells must be carried out in an approved Containment Level 2 (CL2) physical laboratory. This includes, but is not limited to vectors derived from Adenovirus, Adeno-associated virus, lab adapted strains of Vesicular Stomatitis Virus, alpha viruses, measles virus, murine, avian or feline gamma retroviruses (formerly known as type C retroviruses) and herpes simplex virus type I or II. Even though the gamma retroviral vector may be replication defective, endogenous retroviruses residing within the transduced cells *in vitro* or *in vivo* could package the nascent viral RNA as pseudotyped infectious particles. Both amphotropic and xenotropic retroviruses from different species are capable of infecting human cells. Research utilizing replication defective lentiviral vectors must be conducted in a Containment Level 2 (CL2) physical laboratory with the use of Containment Level 3 (CL3) operational practices (commonly termed CL2+). This includes vectors derived from, but not limited to, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). Researchers are strongly encouraged to use self-inactivating lentiviral vectors. These guidelines also apply to *in vivo* work.

Research involving a live replication competent or defective viral vector containing a known oncogene, regardless of the type of the viral vector, requires CL3 if the vector is infectious for human cells. Viral vectors expressing genes that are known to be anti-apoptotic or promote cell survival and/or proliferation may also require higher levels of containment but will have to assessed on a case by case basis by the UWO Biohazards Subcommittee.

It is recognized that experiments involving direct injection of virus or a virus-transduced cell line into an animal place significant burden on the researchers in order to meet the recommended guidelines. For example, conducting a stereotaxic injection of a viral vector into a targeted area of the brain is generally not possible using conventional laminar flow hoods. Whole animal imaging (MRI, CT, PET or ultrasound, bioluminescence) and flow cytometry of live vector-transduced cells are additional examples where biosafety issues make experimental protocols more difficult. In an effort to help reduce this burden, the following procedures are proposed to provide proof that no virus is being released from transduced cells as a way to reduce the need for CL2 or CL2+ containment.

Gamma retrovirus or lentivirus vectors:

For experiments that require that cells stably transduced with a gamma retroviral or lentiviral vector be injected into an animal the level of containment can be dropped providing the following conditions can be satisfied:

1. The use of self-inactivating gamma retroviral or lentiviral vectors is strongly advised when available. Commercially available lentiviral vectors are self-inactivating. Most gamma retroviral vectors are not.

2. Once stable viral transductants have been selected/established under the required containment conditions, the engineered cells containing a reporter gene (GFP or luciferase for example), a gene that mediates targeted recombination (Cre or Flip recombinase) or a gene that modifies metabolism but does not affect the cell cycle or proliferation can be tested for the absence of virus production. This can be demonstrated by taking the clarified cell supernatant from the transduced cell line after 5 to 10 cell passages and adding it to cultures of the original uninfected cells or a similar cell line that is highly permissible to viral infection. Reporter gene assays can then be conducted after 48 to 72 hours of culture. However, these types of assays may not be particularly sensitive and should be discussed with the Biohazard Subcommittee in advance. The preferred approach, and that which must be done for all non-reporter gene constructs, is to use quantitative PCR as the confirmatory assay with appropriate standards to confirm assay sensitivity. The assay must be sensitive enough to detect at least one infected cell per 10^6 uninfected cells. Alternatively, clarified supernatants from cell passage 5 to 10 can be concentrated by ultracentrifugation and the pellet area extracted in the presence of carrier RNA. Real time qRT-PCR can be conducted with standards to determine if virus is being released from the stably transduced cells. In either case one primer should be derived from the vector sequence and the other from the transgene of interest. If the virus is undetectable in either of these assays, a CL2 or CL2+ cell line could be handled at its original, nontransduced containment level. Animals injected with these reclassified cells could also be handled at their original, nontransduced containment levels. If gamma retro virus or lentivirus vectors must be injected directly into animals then injections can be conducted in a level 2 room outside of a laminar flow hood provided appropriate personal protective equipment is worn and appropriate decontamination procedures are in place. Once this proof of principle experiment is conducted and submitted to the Biohazard Subcommittee for review, then all subsequent experiments using the same gamma retroviral or lentiviral vector transduced cells can be done under reduced containment. Positive detection of the virus in culture supernatant or as integrated viral DNA from test cells would require maintenance of the virally transduced containment level.

Note that this "dropdown" option does not apply to immunocompromised mice repopulated with primary human or nonhuman primate (NHP), unmodified primary or viral vector modified primary cells. For those mice, the containment must not be lower than CL2 (the standard for handling any primary human material) or CL2+ (the standard for handling NHP material). If the primary cells are known to be infected with a risk group 3 human pathogen, then they must be handled at the containment level appropriate for that pathogen. If the transduced gene is known to promote cell survival or alter cell cycling in favour of proliferation (as in the case of an oncogene), then CL2+ or a higher containment level, determined by a risk assessment made in collaboration with the Biohazard Subcommittee, must be maintained for live viral vector work, especially if the vectors are capable of infecting human cells.

Adenovirus vectors:

For animal experiments that require the use of replication competent adenovirus vectors (first generation vectors), level 2 containment must be observed regardless of the transgene to be used. For experiments using 2nd or 3rd generation replication defective Adenovirus vectors that do not contain an oncogene or genes that promote cell survival and or cell proliferation, direct injection

of virus infected cells or direct injection of virus can be done outside a laminar flow hood in an approved level 2 room with personal protective equipment worn once the following proof of principle condition has been satisfied:

Following injection of the animal, bodily fluids such as blood, bronchial lavage, and urine as well as stool should be collected at several time points over the first 14 days post-infection. Quantitative PCR with the use of positive spiking controls and assay sensitivity controls can then be used to demonstrate that the recombinant Adenovirus is not being released from the infected animal. Once this proof of principle experiment is conducted then all following experiments using the same Adenovector can be done under reduced containment conditions and the animals can be returned to CL1 animal housing at the point when the Q-PCR gave reproducible negative results.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

Adeno-associated virus vectors:

For experiments using recombinant Adeno-associated virus vectors it is strongly recommended that the vector be generated using a construct that can generate the vector by transfection such that helper virus is not required. For direct animal injection experiments the same proof of principle experiment as described for the Adenovirus vectors must be conducted before lowering of the containment level for animal housing can be considered.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

Other viral vectors:

Experiments requiring the use of less commonly used viral vectors will need to be considered by the Biohazard Subcommittee on a case by case basis in consultation with AUS-ACVS.

Certificate of Analysis

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PRODUCT: Y2HGold Yeast Strain

CATALOG No. 630498

LOT NUMBER
Specified on product label.

STORAGE CONDITIONS
–70°C

SHELF LIFE
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS
Dry ice (–70°C)

DESCRIPTION
The *Saccharomyces cerevisiae* strain Y2HGold is designed for use with Clontech's Matchmaker™ Gold Yeast Two-Hybrid System. Y2HGold includes the sensitive Aureobasidin A antibiotic resistance gene as one of four reporters—providing exceptionally stringent yeast two-hybrid (Y2H) screening. This strain contains distinct *ADE2, HIS3, MEL1,* and *AUR1-C* reporter constructs that are only expressed in the presence of GAL4-based protein interactions. Y2HGold is extremely effective in minimizing false positive protein interactions and background during a typical GAL4-based two-hybrid screen.

PACKAGE CONTENTS
- 0.5 ml *Saccharomyces cerevisiae* Y2HGold
- Matchmaker Gold Yeast Two-Hybrid System User Manual (PT4084-1)

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

- The nutritional requirements (i.e., auxotrophic markers) of Y2HGold Yeast Strain were verified by streaking samples onto six types of SD minimal media: SD/-Ade, SD/-His, SD/-Leu, SD/-Trp, SD/-Ura, and SD/-Met.
- Reporter function. The function of the *ADE2, HIS3, MEL1* and *AUR1-C* reporters in Y2HGold Yeast Strain were verified using positive and negative control plasmids and plating on minimal media lacking adenine and histidine, and containing X-alpha-Gal and Aureobasidin A.

APPROVED BY: _____

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10-16-2007, 07:35 AM #1

Simon Holdaway Posts: n/a

Bacterial Transformation Strains

Bacterial Transformation Strains

There are three major strains of E.coli used in current transformation experiments. All are derived from the original, non-pathogenic K12 strain. Many practitioners of transformation experiments do not fully appreciate the importance of the genotypes of these strains and the impact their choice of strain can have on downstream applications for their DNA.

DH5alpha

The E.coli strain DH5alpha is the most common strain used for transformations in research laboratories. Its full genotype is:

F- endA1 glnV44 thi-1 relA1 gyrA96 deoR nupG lacZdeltaM15 hsdR17

The full details of the DH5alpah genome are given at the end of this article, but the major points of interest are that the strain is endA1 ? which allows for clean DNA preparations from plasmid mini-preps, and that it lacks the alpha portion of the lacZ gene and thus can be used for blue-white screening. DH5alpha is also resistant to nalidixic acid.

HB101

Most of you are familiar with the HB101 strain because it is the E.coli strain used by Bio-Rad for their ubiquitous pGLO transformation kit. The full genotype is:

F- mcrB mrr hsdS20(rB-mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20 glnV44

The strain is a hybrid of E.coli strains K12 and E.coli B and is the best host for pBR322. Biorad uses this strain because it is unable to metabolize arabinose. The GFP gene on the pGLO plasmid is under the control of an arabinose promoter. Adding arabinose turns on the promoter. If the E.coli could metabolize the arabinose sugar then the promoter would eventually turn off as arabinose disappeared from the cell. Thus GFP expression would be lost. HB101 lacks the endA1 genotype, which means plasmid mini-preps from this strain will be lower yield and less ?clean? due to the action of a non-specific DNA endonuclease that HB101 produces, but DH5alpha does not. Interestingly, HB101 is also resistant to streptomycin, so don?t use that antibiotic as a selective marker in your transformation experiments!

JM109

The final ?common? transformation strain is JM109, a strain originally developed for cloning repetitive

DNA. Some transformation educational kits on the market still use this strain. The genotype is:

endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ delta(lac-proAB) e14- [F? traD36 proAB+ lacIq lacZdeltaM15] hsdR17

The important things to know about JM109 are that it is good for cloning and downstream DNA applications (no endonuclease activity). It is nalidixic acid resistant and allows cloning of unmethylated DNA from PCR reactions. It can be used for blue-white screening, unlike HB101, but most scientists prefer the DH5alpha strain

DH5alpha genome explained:

DH5 alpha: F- endA1 glnV44 thi-1 relA1 gyrA96 deoR nupG lacZdeltaM15 hsdR17

F- does not carry the F+ plasmid for conjugation. An important step in ensuring biosafety.

endA1 does not produce Endonuclease I. This allows cleaner plasmid preps since you avoid non-specific digestion of plasmid DNA.

glnV44 suppression of the amber (or UAG) stop codon and replacement with glutamine. This is required for some bacteriophage growth.

Thi-1 requires thiamine

relA1 permits production of RNA without protein synthesis

gyrA96 nalidixic acid resistance ? conferred by a mutation on DNA gyrase

deoR permits uptake of larger sized plasmids

nupG also allows uptake of large plasmids

lacZdeltaM15 deletion of the alpha portion of the beta-galactosidase gene. Allows blue-white screening

hsdR17 allows cloning of unmethylated DNA ? mostly from PCR reactions

HB101 genome explained

F- mcrB mrr hsdS20(rB-mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20 glnV44

The strain is a hybrid of E.coli strains K12 and E.coli B and is the best host for pBR322.

F- does not carry the F+ plasmid for conjugation. An important step in ensuring biosafety since it prevents accidental dissemination of plasmids.

mcrB eliminates restriction of DNA at the sequence R(m)C

mrr eliminates restriction of DNA at the sequence C(m)Ag or G(m)AC

hsdS20(rB-mB-)

recA13 reduces recombination in cloned DNA, UV sensitive because of DNA repair deficiency

leuB6 requires leucine

ara-14 unable to metabolize arabinose

proA2 requires proline

lacY1 deletion of lactose permease (lacY) from lac operon

galK2 unable to metabolize galactose

xyl-5 unable to metabolize xylose

mtl-1 unable to metabolize mannitol

rpsL20 mutation in ribosome, conveying streptomycin resistance

gln44 suppression of the amber (or UAG) stop codon and replacement with glutamine. This is required for some bacteriophage growth.

JM109

endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ delta(lac-proAB) e14- [F? traD36 proAB+ lacIq lacZdeltaM15] hsdR17

endA1 no endonuclease I activity. Allows for cleaner DNA preparations.

glnV44 suppression of amber (UAG) stop codons by insertion of glutamine

thi-1 requires thiamine

relA1 relaxed phenotype which allows mRNA synthesis without protein synthesis

gyrA96 nalidixic acid resistant

recA1 reduces recombination in cloned DNA, UV sensitive because of DNA repair deficiency

mcrB+ no restriction at methylated RmC sequences

delta(lac-proAB)

e14- contains a prophage remnant which contains the mcrA gene

[F? traD36 proAB+ lacIq lacZdeltaM15] carries an F plasmid (for conjugation) carrying these chromosomal genes

hsdR17 required for transformation of unmethylated DNA (from PCR rxns)

« Previous Thread | Next Thread »

Posting Rules

pSUPER RNAi System™

VECTOR: pSUPER.neo
CATALOG#: VEC-PBS-0003/0004

Length: 4699 bp

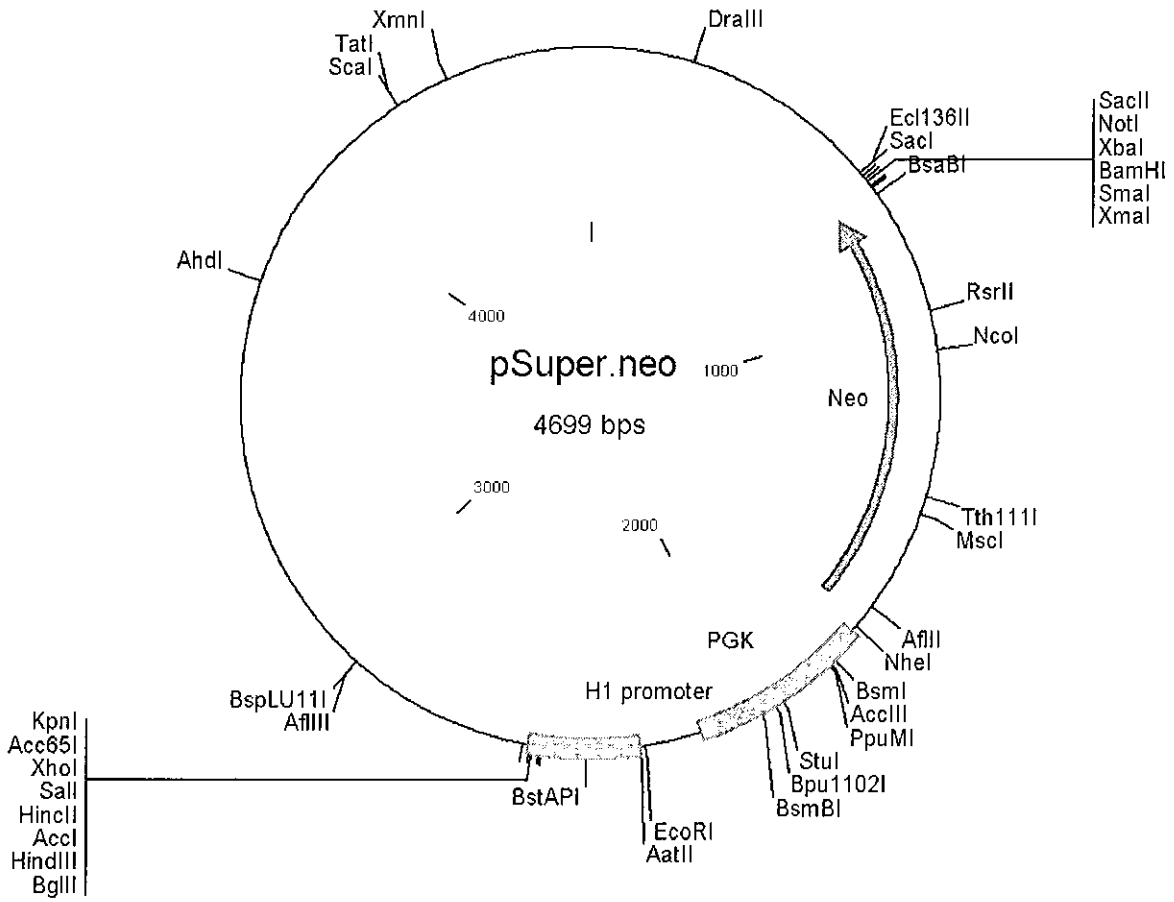
Key Sites

BglII: 2451
HindIII: 2457
EcoRI: 2230
SalI: 2472
XhoI: 2478

Vector Features

f1(+) origin: 135-441
PGK promoter: 2110-1712
Neo ORF: 1684-715
H1 promoter: 2235-2483
Ampicillin resistance ORF: 3714-4193

T7 primer binding site (AATACGACTCACTATAG): 627-643
T3 primer binding site (CTTTAGTGAGGGTTAAT): 2512-2528
M13(-20) primer binding site (GTAAAACGACGGCCAGT): 600-616
M13 reverse primer binding site (CATGGTCATAGCTGTT): 2546-2561



Clontech

MSCV Retroviral Expression System

You may also be interested in these Clontech products:

Lenti-X™ Vector Overview
Fluorescent Lentiviral Expression Vectors
Viral Vectors for Fluorescent Protein Delivery
Fluorescent Retroviral Vectors
Lentiviral Inducible Expression Systems
Lenti-X™ Expression Systems
Lenti-X™ HT Packaging Systems

The MSCV (Murine Stem Cell Virus) Retroviral Expression System contains vectors that are optimized for introducing and expressing target genes in pluripotent cell lines, including murine or human hematopoietic, embryonic stem (ES), and embryonal carcinoma (EC) cells. They can also be used effectively with any mammalian cell line (1–3). This highly efficient system is ideal for analyzing gene function in development, embryogenesis, or immune response, in both cell culture and transgenic assays.

Designed for Difficult-to-Infect Cells

The MSCV System contains three vectors: pMSCVneo, pMSCVhyg, and pMSCVpuro. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell PCMV virus that allows you to work with hard-to-transduce cell lines. This LTR differs from the MMLV LTR by several point mutations and a deletion that together enhance transcriptional activation and prevent transcriptional suppression in ES and EC cells. As a result, the PCMV LTR drives high-level, constitutive expression of the target gene in stem cells or other mammalian cell lines. The MSCV System includes the RetroPack™ PT67 Packaging Cell Line, which produces high-titer virus able to infect a broad range of mammalian host cells.

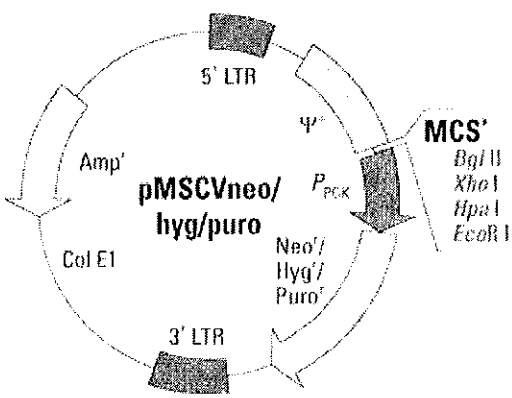


Figure 1. pMSCV Vectors map.

References

1. Hawley, R. G., *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:10297–10302.
2. Keller, G., *et al.* (1998) *Blood* **92**:877–887.
3. Hawley, R. G., *et al.* (1994) *Gene Ther.* **1**:136–138.

Components

pMSCVneo Vector

Storage Conditions

–196°C for RetroPack PT67 Cell Line

Cell Biology

ATCC® Number:	**CCL-2™** Order this Item	Price:	**$256.00**

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [CELLS CONTAIN PAPOVAVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
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 ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18

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Cytogenetic Analysis:	Modal number = 82; range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of Ml, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
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:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	

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.
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:	

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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]

The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

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: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Cell Biology

ATCC® Number: **CCL-247™** Order this Item Price: **$256.00**

Designations:	HCT 116
Depositors:	MG Brattain
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** colon **Disease:** colorectal carcinoma
Cellular Products:	carcinoembryonic antigen (CEA) 1 ng per 10 exp6 cells per 10 days; keratin
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
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 7,10 D13S317: 10,12 D16S539: 11,13 D5S818: 10,11 D7S820: 11,12 THO1: 8,9 TPOX: 8,9 vWA: 17,22
Cytogenetic Analysis:	The stemline chromosome number is near diploid with the modal number at 45 (62%) and polyploids occurring at 6.8%. The markers 10q+ and t(?8p;18q) are present in all metaphases and t(9q;?16p-), in 80% of the cells karyotyped. N16 is monosomic in the presence of, but disomic in the absence of t (9q;?16p-). N10 and N18 are monosomic and other chromosomes from those mentioned above are disomic. Q-band observations revealed the presence of the Y chromosome, but not in all cells (50% of cells lacked the Y in G-band karyotypes).

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Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1 PGM1, 1 PGM3, 1
Age:	adult
Gender:	male
Comments:	The cells are positive for keratin by immunoperoxidase staining. HCT 116 cells are positive for transforming growth factor beta 1 (TGF beta 1) and beta 2 (TGF beta 2) expression. This line has a mutation in codon 13 of the ras protooncogene, and can be used as a positive control for PCR assays of mutation in this codon.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** Growth and plating efficiency are enhanced by using a feeder layer of murine fibroblasts.
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (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. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase