THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 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	**Dr. Shawn Li**
DEPARTMENT	**Biochemistry**
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EMAIL	**sli@uwo.ca**

Location of experimental work to be carried out: Building(s) **SDRI** Room(s) **108A**

*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: **Canada Cancer Society**
GRANT TITLE(S): **Numb as a Tumor Suppressor**
List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
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Gurpreet Dhami	**gdhami2@uwo.ca**	**Nov 9, 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.

1. E. coli DH5α was from Invitrogen. E. coli BL21 was from Novagen. E. coli DH5α and BL21 strains are used as competent cell for transformation. They are stored at -80°C freezer. When using for transformation, they will be thawed to 4°C on ice, then take 100 µl of E. coli to mix with 10 ng plasmid DNA for 20 min on ice, hot shock at 42°C for 90 seconds, stand on ice fro 2 min. Add 1 ml of LB medium culture at 37°C for 1 hr, take 200 µl of mixture on antibiotic LB culture plate at 37°C overnight. The colonies will be used for protein or DNA purification. Waste E. coli, liquids and containers will be treated by bleach to decontaminate. All containers or tubes will be washed and autoclaved. All autoclave treated stuff will be disposed as regular garbage.

2. Cell lines HEK293, P19 and MDCK2 were from ATCC. HEK293, P19 and MDCK2 are used as experimental models. They are stored in liquid nitrogen. When using for experiments, they will be thawed to 37°C in water bath quickly, then put them into culture plate with 10% FBS completed medium to grow up at 37°C for 2 days or so. All operation will be preceded in class II biological safety cabinet in containment level 2 culture room. Then aspirate culture medium from plates. Briefly rinse the cell layer with PBS to remove all traces of serum. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to plate and observe cells under an inverted microscope until cell layer is dispersed. 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. Incubate cultures at 37°C for 1-2 days. Cells will be treated by different reagents and methods depending on each research purpose. Waste culture medium and cells will be collected into bleach containing waste bottle to decontaminate. All used experiment materials (cultural plates, pipets, tips, etc.) will be collected and autoclaved. All autoclave treated stuff will be disposed as regular garbage.

Please include a one page research summary or teaching protocol.

Numb is a protein originally identified for its role in animal development. However, it was recently found to also act as a tumor suppressor which regulates cell growth, cell-cell adhesion, and cell migration. In breast and prostate cancer cells, mutation of the *numb* gene or loss of the protein correlates with poor prognosis.

Our lab has identified a new function for Numb in regulating cell-cell adhesion and epithelial-to-mesenchymal transition (EMT) - a critical step in cancer progression in which cancer cells lose adhesiveness and orientation and migrate away from the original site of lesion to invade surrounding tissues and remotes sites.

Work from our lab and others make us to believe that Numb a multi-faceted proteins that plays an important role in the onset and progression of cancers by regulating cell growth, survival, orientation and cell-cell adhesion.

As such, it represents a druggable target for cancer treatment. In this current proposal, we wish to characterize these diverse functions of Numb using a combination of biochemical, biophysical and cellular methods.

Specifically, we will examine a dynamic interplay between Numb and tyrosine kinases, enzymes in the body that modify other proteins by adding a phosphate group. Excessive tyrosine kinase activity is often associated with cancer. Moreover, we will determine the mechanism behind a role for Numb in regulating cell-cell adhesion, cell orientation and movement. And finally, we will characterize an interaction between Numb and Alk, a kinase that is aberrantly activated in a wide spectrum of different cancers, and elucidate how this interaction affects cell growth and death.

It is anticipated that these studies will provide novel insights into Numb function in cancer and aid in efforts to develop cancer therapies that specifically target Numb or its binding partners.

Numb interacting protein (NIP) is another protein of interest in our lab. NIP is involved in the production of reactive oxygen species (ROS), and its overexpression in stem cells enhances neuronal and cardiac differentiation. We have linked some of this phenotype to enhanced ROS production, but both our protein's cellular localization and its amino acid sequence suggest other roles.

We believe this protein may work as a co-activator for a variety of hormones and transcription factors. We are interested in determining whether overexpression of our protein of interest will enhance luciferase production in both the pGL3-NFAT and pGL3-RARE reporter vectors. We have selected these particular reporters since NFAT has previously been shown to be stimulated by ROS. We have selected the retinoic acid response element (RARE) because our protein of interest stimulates differentation in the absence of retinoic acid induction, and we believe it may be acting as a co-activator for the retinoic acid receptor. We are planning to transfect 293 cells with these reporter vectors and our protein of interest in order to start testing our hypotheses. We hope that our work will identify new roles for this protein.

ADAMTS12 is a protease that cleaves proteins in response to cell signaling thereby changing the cells response to specific signals.

We recently see co-purified ADAMTS12 with a transcription factor TLE4. TLE4 can act as an activator of transcription and when cleaved, by an unknown protease; a transcriptional repressor. Since ADAMTS12 is a protease and can bind to and interact with TLE4 we speculate that ADAMTS12 is in fact the protein responsible for cleavage of TLE4.

We will test this hypothesis through standard Li laboratory protocols involving transient transfection of HEK293 cells with ADAMTS12 or a enzymatically inactive form of ADAMTS12. We will then compare the size of TLE4 by standard Li laboratory immunoblot techniques.

Previous studies have shown that TLE4 not only changes size but also has different co-factors in the cleaved form. We will evaluate the effect of ADAMTS12 through both assaying of TLE4 size by immunoblot but also through determining the proteins associated with TLE4 and the ratio of known exclusive cleaved interactors such as KDM5b.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? **O YES**
(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 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	**O No**	**O No**	**O No**			**O 1**
E. coli BL21	**O No**	**O No**	**O No**			**O 1**
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes 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? **O YES**
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 No**		Not applicable
Rodent	**O No**		
Non-human primate	**O No**		
Other (specify)	**O No**		

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

Product code 18258
Product name ME DH5A COMPETENT CELLS

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	3-7

3. HAZARDS IDENTIFICATION

Emergency Overview

Irritating to eyes. Irritating to skin. Components of the product may be absorbed into the body through the skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
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: Eyes. Skin.

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
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	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

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 189	°F No data available
Melting point/range	°C 18.4	°F No data available
Flash point	°C 94	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Irritating to eyes.
Skin	Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation	May cause irritation of respiratory tract.
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 — Eyes. Skin.

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

Proper shipping name Not classified as dangerous within the meaning of transport regulations

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

Novagen®

Order Number

Customer Number

1. Product and Company Identification

Supplier: Manufactured by EMD Biosciences, Inc.
441 Charmany Drive
Madison, WI 53719
(608)238-6110
(800)207-0144
FAX: (608)238-1388

P.O. Box 12087
La Jolla, CA 92039-2087
(858)450-5558
(800)854-3417
FAX: (858)453-3552

Catalog #: 69449

In Case of Emergency:

Product Name: BL21 Competent Cells

2. Composition and Information on Ingredients

Ingredient Name	CAS No.	Product No.	EC Symbol	R-Phrases
Calcium Chloride	10043-52-4	RC0030	Xi	R36

Note: See section 8 for occupational exposure limits and section 11 for LC50/LD50 information.

3. Hazards Identification

Primary Hazards and Critical Effects	:	RC0030	CAUTION! BE HARMFUL IF SWALLOWED. MAY CAUSE EYE IRRITATION. Avoid contact with eyes. Do not ingest. Wash thoroughly after handling.
Specific Chemical Hazards	:		Not applicable.
Human Health Hazards	:	RC0030	Irritating to eyes.
Environmental Hazards	:		Not applicable.

MAY

4. First Aid Measures

Inhalation	:	RC0030	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion	:	RC0030	Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately.
Skin Contact	:	RC0030	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
Eye Contact	:	RC0030	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.
Notes to Medical Doctor	:		Not available.

5. Fire-Fighting Measures

Extinguishing Media	:	Use foam or all purpose dry chemicals to extinguish.
Fire-Fighting Procedures	:	Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.
Unusual Explosion Hazards	:	Not applicable.
Hazardous Decomposition Products	:	Not available.

6. Accidental Release Measures

Personal Precautions	:	Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (Section 8). Follow all fire fighting procedures (Section 5).
Environmental Precautions and Clean-up Methods	:	If emergency personnel are unavailable vacuum or carefully scoop up spilled materials and place in an appropriate container for disposal. Avoid creating dusty conditions and prevent wind dispersal. Minimize contact of spilled material with soils to prevent runoff to surface waterways.

Note: See section 1 for emergency contact information and section 13 for waste disposal.

7. Handling and Storage

Handling	:	RC0030	Avoid contact with eyes. Do not ingest. Wash thoroughly after handling.
Storage	:	Keep container tightly closed. Keep container in a cool, well-ventilated area.	
Packaging Materials	:	Use original container.	

8. Exposure Controls and Personal Protection

Occupational Exposure Limits

Ingredient Name	Occupational Exposure Limits
RC0030	Not available.

Engineering Controls	:	RC0030	No special containment is required. Local exhaust ventilation should be provided.
Personal Protective Equipment			
Respiratory System	:	RC0030	Use an approved, properly fitted, HEPA filter cartridge respirator, or a respirator of greater protection if there is the potential to exceed the exposure limit(s).
Skin and Body	:	RC0030	Work uniform or laboratory coat.
Hands	:	RC0030	Use chemical resistant, impervious gloves. Additional body garments should be used based upon the task being performed (e.g., sleevelets, apron, gauntlets, disposable suits). Appropriate techniques should be used to remove potentially contaminated clothing.
Eyes	:	RC0030	Safety glasses. Goggles, face shield, or other full-face protection if potential exists for direct exposure to dust.

9. Physical and Chemical Properties

Kit Components

0.4 ml Cells
69318: 1 x 10 ul Test Plasmid
69319: 2 x 2 ml SOC Medium
69449: 2 x 0.2 ml BL21 Competent Cells, containing Calcium Chloride (RC0030)

1.0 ml Cells
69318: 1 x 10 ul Test Plasmid
69319: 4 x 2 ml SOC Medium
69449: 5 x 0.2 ml BL21 Competent Cells, containing Calcium Chloride (RC0030)

Flash Point

Not available.

10. Stability and Reactivity

Stability	:	RC0030	The product is stable.
Conditions and Materials to Avoid	:	RC0030	Reactive with acids.
Hazardous Decomposition Products	:		Not available.

11. Toxicological Information

Toxicity Data

Ingredient Name	Test	Result	Route	Species
RC0030	LD50	1000 mg/kg	Oral	Rat
	LD50	1940 mg/kg	Oral	Mouse

Routes of Entry : Eye contact.

Acute Effects

Inhalation	:		Not available.
Ingestion	:	RC0030	Harmful if swallowed.
Skin Contact	:		Not available.
Eye Contact	:	RC0030	Moderately irritating to the eyes.

Chronic Effects

Adverse Effects	:	Not available.
Target Organs	:	Not available.
Carcinogenic Effects	:	Not available.
Mutagenic Effects	:	Not available.
Developmental and Teratogenic Effects	:	Not available.
Reproductive Effects	:	Not available.

Other Information : RC0030 Repeated or prolonged exposure is not known to aggravate medical condition.

12. Ecological Information

Ecotoxicity Data

Ingredient Name	Species	Period	Result
RC0030	Not available.	Not available.	Not available.

13. Disposal Consideration

Waste Handling and Disposal : Waste must be disposed of in accordance with federal, state and local environmental control regulations.

14. Transport Information

Air

IATA-IDGR Class : Not controlled under IATA.

Packing Group

15. Regulatory Information

EU Regulations

Hazard Symbols	:	Xi
Risk Phrases	:	R36- Irritating to eyes.
Safety Phrases	:	S24- Avoid contact with skin.

US Regulations

Hazcom Standard	:	Not controlled under the HCS (United States).
TSCA	:	Not available.
State		Not available.

Catalog #: 69449

Canadian Regulations

WHMIS	:	Not controlled under WHMIS (Canada).
CEPA	:	No products were found.
Provincial	:	No products were found.

16. Other Information

Validated by jcw on 7-15-2003.

Version	:	1.0
Date of Printing	:	8/28/2003.

Notice to Reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above named supplier nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
*Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist. **This product is intended for research use only.***

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. [illegible]		
Applications	[illegible] testing [illegible] transfection host (Nucleofection technology from Amaxa) [illegible] GENCO Transfection Reagent [illegible] testing [illegible]		
Receptors	[illegible] expressed		
Tumorigenic	Yes		
DNA Profile (STR)	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 TH01: 7,9.3 TPOX: 11 vWA: 16,19		
Cytogenetic Analysis	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2%. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The markers 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. [23496] The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment 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	**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. An inoculum of 2 X 10[illegible] to 6 X 10[illegible] [illegible] is recommended. 6. Incubate cultures at 37 °C. Subculture when cell concentration is between [illegible] X 10[illegible] cells/cm2. **Subcultivation Ratio:** 1:10 to 1:20 weekly **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		

Cell Biology

ATCC® Number	CRL-1825™	Order this Item	Price $275.00
Designations	P19		
Depositor	MW McBurney		
Biosafety Level	1		
Shipped	frozen		
Medium & Serum	Not Propagated		
Growth Properties	adherent		
Organism	Mus musculus		
Morphology	epithelial		

Source
Strain: C3H/He
Organ: embryo
Disease: NCCarcinoma, embryonal carcinoma

Restrictions
In addition to the MTA mentioned above, other ATCC terms and conditions may also apply. For the terms of this ATCC material, anyone purchasing ATCC material is solely responsible for obtaining the permits. Please click here for additional information regarding the specific requirements for shipments to your location.

Antibiotic Info
Transduction with N-(phosphonacetyl)-L-aspartate (PALA) resistance

Cytogenetic Analysis
2n = 40, XY (100%)

Comments
The P19 line was derived from an embryonal carcinoma induced in a C3H/He mouse. [2792]
The cells may be cloned at high efficiency in medium containing 0.3% agar. [2792]
The cells are tumorigenic.
The cells can be induced to differentiate into neural and glial-like cells in the presence of 0.5 µM retinoic acid. [27492]
In the presence of 1% dimethylsulfoxide (DMSO), the cells differentiate to form cardiac and skeletal muscle-like elements, but do not form neural or glial-like cells. [27513]
In the presence of both DMSO and retinoic acid, the cells differentiate as in the presence of retinoic acid alone. [27513]

Propagation
ATCC complete growth medium: The base medium for this cell line is Alpha Minimum Essential Medium with ribonucleosides and deoxyribonucleosides. To make the complete growth medium, add the following components to the base medium: bovine cell serum to a final concentration of 7.5%, fetal bovine serum to a final concentration of 2.5%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

Subculturing
Protocol: Do not allow the cells to become confluent.

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 7.0 mL of complete growth medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A 1:3 to 1:6 split ratio is recommended.
Medium Renewal: Add fresh medium as needed every 48 hours.

Preservation
Freeze medium: Complete growth medium, 90%; DMSO, 10%
Storage temperature: liquid nitrogen vapor phase

Cell Biology

ATCC® Number	CRL-2936™ Order this Item	Price	$355.00
Designation	MDCK.2		
Depositor	Dr. Piet B. Gaytano and Dr. Edward Adrian, ATCC		
Biosafety Level	1		
Shipping	frozen		
Morphology & Growth	See Propagation		
Growth Properties	adherent		
Organism	Canis familiaris		
Morphology	epithelial		

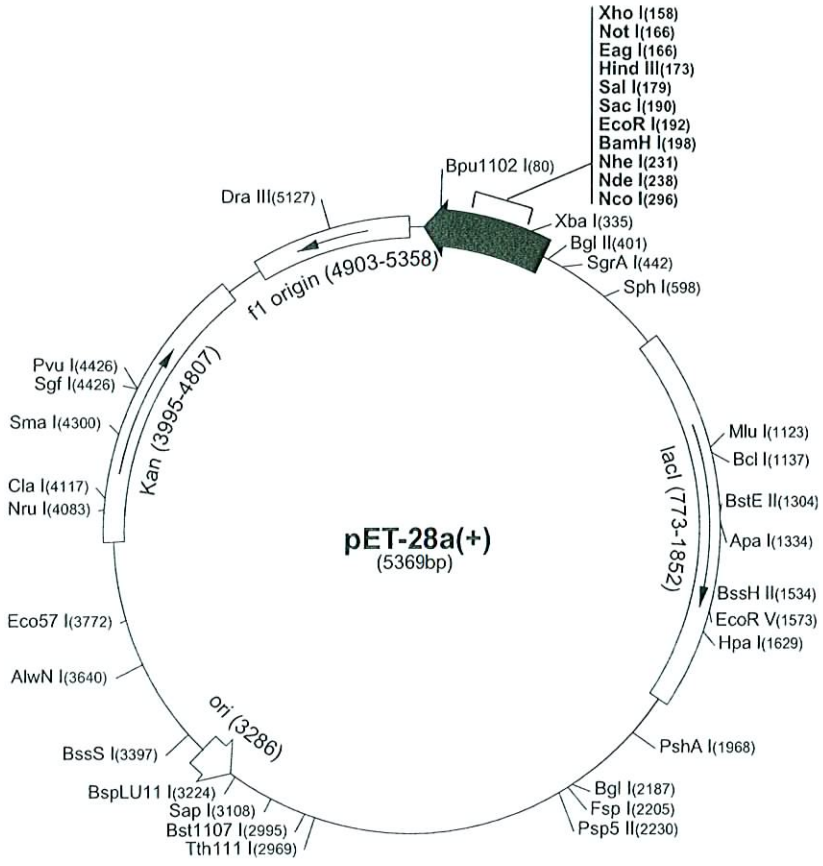
Source	**Organ:** kidney (cortex and medulla) **Disease:** normal
Permits/Forms	In addition to U.S. EPA maintenance permit, other ATCC quality management permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is responsible for obtaining the permits. Please call us for information regarding the specific requirements for shipment to your location.
Molecular	Isolated: March 1987
Virus Susceptibility	Influenza A virus Influenza B virus
Antigen Expression	Epithelial cadherin (E-cadherin): cell adhesion molecule, expressed Zona occludens (ZO-1): tight junction protein, expressed Alkaline phosphatase (tissue-specific isozyme): not expressed Cytokeratin (CK): 4, 5, 6, 8, 10, 13, 15, 18: expressed Hepatocyte nuclear factor 3 alpha 2 (HNF-3 alpha 2): detected Vimentin: expressed
Cytogenetic Analysis	Hypodiploid cell line with a modal chromosome number of 61 with low polyploidy ratio. Several unidentified marker chromosomes were present in most of the cells examined.
Age	adult
Comments	Cell line was derived by cloning (limited dilution) the parental cell line MDCK (ATCC® CCL-34). This cell line is susceptible to a wide range of influenza virus and is sustained replication of *C. psittacosis*.
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%.
Subcultivation	**Protocol:** Volumes used in this protocol are for 75 sq cm flasks, proportionally reduce or increase volumes of dissociation medium for culture vessels of other sizes.

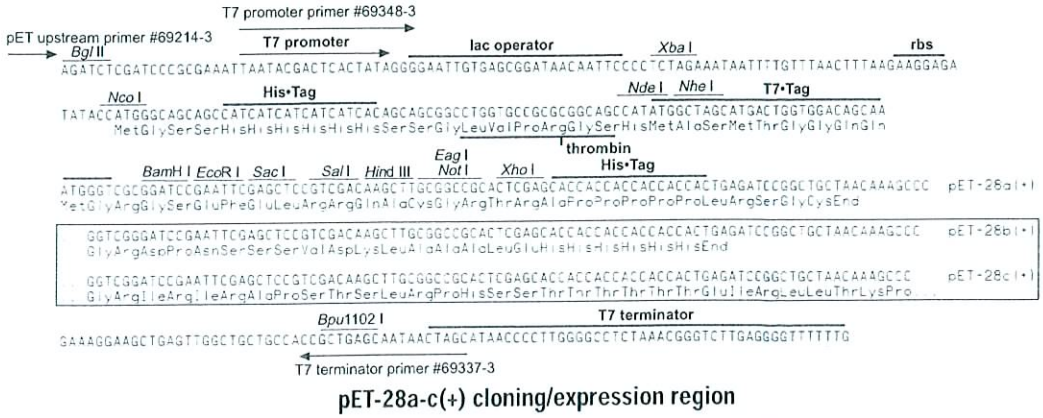
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) (0.25% (w/v) trypsin - 0.53 mM EDTA solution is recommended) to remove all traces of serum which contains trypsin inhibitor.
3. Add 1.0 to 2.0 mL of trypsin-EDTA solution 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 37°C to facilitate dispersal.
4. Add 6.0 to 9.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 1 X 10^4 to 2 X 10^4 viable cells/sq cm is recommended.
6. Incubate cultures at 37°C. Subcultivate when the cell concentration is between 7 X 10^4/mL and 1 X 10^5 cells/mL.
   Subcultivation ratio: A subcultivation ratio of 1:2 to 1:8 is recommended.
   Medium renewal: Every 2 to 3 days.

Preservation	**Freeze medium:** Complete growth medium (ATCC 30-2003) + 10% (v/v) DMSO Liquid nitrogen vapor phase
Doubling Time	Approximately 27 hours

pET-28a(+) sequence landmarks	
T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites (*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.





pET-28a-c(+) cloning/expression region

pGEX-2T (27-4801-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Gly Ile His Arg Asp
CTG GTT CCG CGT GGA TCC CCG GGA ATT CAT CGT GAC TGA CTG ACG

BamH I Sma I EcoR I Stop codons

pGEX-2TK (27-4587-01)

Thrombin Kinase

Leu Val Pro Arg↓Gly Ser Arg Arg Ala Ser Val
CTG GTT CCG CGT GGA TCT CGT CGT GCA TCT GTT GGA TCC CCG GGA ATT CAT CGT GAC TGA

BamH I Sma I EcoR I Stop codons

pGEX-4T-1 (27-4580-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Glu Phe Pro Gly Arg Leu Glu Arg Pro His Arg Asp
CTG GTT CCG CGT GGA TCC CCG GAA TTC CCG GGT CGA CTC GAG CGG CCG CAT CGT GAC TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-4T-2 (27-4581-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Gly Ile Pro Gly Ser Thr Arg Ala Ala Ala Ser
CTG GTT CCG CGT GGA TCC CCA GGA ATT CCC GGG TCG ACT CGA GCG GCC GCA TCG TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codon

pGEX-4T-3 (27-4583-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Asn Ser Arg Val Asp Ser Ser Gly Arg Ile Val Thr Asp
CTG GTT CCG CGT GGA TCC CCG AAT TCC CGG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

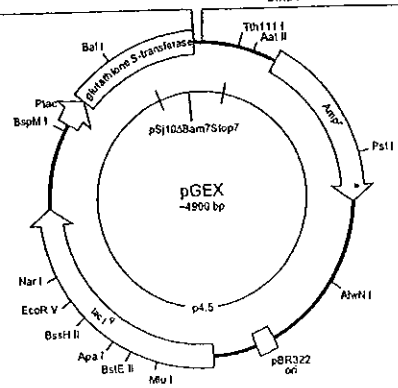
BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-6P-3 (27-4599-01)

PreScission™ Protease

Leu Glu Val Leu Phe Gln↓Gly Pro Leu Gly Ser Pro Asn Ser Arg Val Asp Ser Ser Gly Arg
CTG GAA GTT CTG TTC CAG GGG CCC CTG GGA TCC CCG AAT TCC CGG GTC GAC TCG AGC GGC CGC

BamH I EcoR I Sma I Sal I Xho I Not I



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	O **Yes**	**HEK293**	**ATCC**
Rodent	O **No**		
Non-human primate	O **No**		
Other (specify)	O **Yes**	**P19, MDCK2**	**ATCC**

*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 **2**

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O **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 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? O **YES** If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O **YES**

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or transfection
E. coli DH5α	pET-28a	Novagen	No	Transformation to get His-tag fusion protein or GST-fusion protein
E. coli BL21	pGEX-4T3	GE Healthcare	No	

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

4.3 Will genetic modification(s) involving viral vectors be made? O **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 **NO**
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O **NO**
- SV 40 Large T antigen O **NO**
- E1A oncogene O **NO**
- Known oncogenes O **NO**
- Other human or animal pathogen and or their toxins O **NO**

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

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O **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** If no, please proceed to section 7.0

6.2 Name of animal species to be used **C57/BL6 and Balb/C wild type mice.**

6.3 AUS protocol # **2008-033-01**

6.4 Will any of the agents listed in section 4.0 be used in live animals O **NO**

6.5 Will the agent(s) be shed by the animal: O **YES**

_____

_____

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 **No** If no, please proceed to section 8.0

7.2 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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O **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 **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 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? **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 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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 **O 2**

13.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus_____

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: Jan 14, 2011

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
Using 70% Alcohol or Bleach to reduce any risks from spill or contamination._____

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

1. Report to PI; 2. Call 911 or UCC to get medical emergency treatment at once; 3. Report to safety officer in UWO if necessary._____

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: