

Modification Form for Permit BIO-UWO-0064

Permit Holder: Chil Kang

Approved Personnel

(Please stroke out any personnel to be removed)

- Hwayong An
- Kunyu Wu
- Elizabeth Banaskowska
- ~~Chad Michalski~~
- Rajl Singh

Additional Personnel

(Please list additional personnel here)

Cyoung Kim

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Vesicular stomatitis virus (lab adapted), Adeno virus, E. Coll (DH 5 alpha, xL10 Gold)	
Approved Cells	Human (established): A549, Hep G2. Rodent (established): Baby hamster kidney, Chinese hamster ovary. Non-human Primate (established): Vero	3D4/21 - Pig alveolar MA ZBTx - Bovine trachea cell E. Derm - horse skin cell
Approved Use of Human Source Material		
Approved GMO	Transcription vector (pTV), HCV genes inserted into VSV genome, Adeno virus serotype 5 replaced by HIV Gag gene	
Approved use of Animals	Mouse	

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 4, 2008

Signature of Permit Holder: *Chil Kang*

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

A brief description of the cell lines and the work that requires the cell lines

We will use 3D4/21 cells (Pig alveolar macrophage cell line), EBTr cells (Bovine tracheal cell line), and E. Derm cells (Horse skin cell line) to test infectivity of vesicular stomatitis virus.

1. 3D4/21 cells are pig alveolar macrophages, which were immortalized with SV40 large T antigen. They belong to BSL II.
2. EBTr cells are cow embryonic tracheal cells, which belong to BSL I.
3. E. Derm cells are horse skin cell originally isolated from a 4 year old female quarterhorse. E. Derm cells belong to BSL I.

Cell Biology

ATCC® Number: **CCL-57™** Order this Item Price: **\$417.00**

Designations: E. Derm (NBL-6) Depositors: AJ Kniazeff, WA Nelson-Rees, NB Darby

Biosafety Level: 1 Shipped: frozen

Medium & Serum: See Propagation Growth Properties: adherent fibroblast

Organism: Equus caballus (horse) Morphology:

Source: **Organ:** skin
Strain: quarterhorse
Tissue: dermis

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** September, 1965

Virus Susceptibility: herpes simplex; reovirus 3; vesicular stomatitis (Ogden); vaccinia

Virus Resistance: adenovirus 5; coxsackievirus A9, B5; poliovirus 2

Reverse Transcript: negative

Cytogenetic Analysis: model number = 64; range = 52 to 145. The donor's sex (female) is confirmed chromosomally. Although the chromosome numbers vary widely, 55% of the cells have a chromosome number within + or -1 of the diploid and modal number (64).

Age: 4 years

Gender: female

Comments: Cells appear to senesce after 40 passages.

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.
Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: Two to three times weekly

Preservation: **Freeze medium:** Complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor temperature

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

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Cell Biology

ATCC® Number: **CCL-44™** Order this Item Price: **\$399.00**

Designations: **EBTr (NBL-4)** Depositors: **AJ Kniazeff, WA Nelson-Rees, NB Darby**

Biosafety Level: **1** Shipped: **frozen**

Medium & Serum: See Propagation Growth Properties: **adherent**

Organism: **Bos taurus (cow)** Morphology: **fibroblast**

Source: **Organ:** trachea
Disease: normal

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Virus Susceptibility: **bovine viral diarrhea; infectious bovine rhinotracheitis; parainfluenzavirus 3; herpes simplex; reovirus 3; vesicular stomatitis (Ogden); vaccinia**

Virus Resistance: **human adenovirus 5; poliovirus 2; coxsackievirus A9, B5**

Reverse Transcript: **negative**

Age: **embryo**

Gender: **male**

Comments: **The cells are negative for bovine viral diarrhea virus (BVDV). [22883]**

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

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended

Medium Renewal: Twice per week

Subculturing: **Remove medium, and rinse with 0.25% trypsin, 0.03% 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.

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

References: **22883: Bolin SR, et al. Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. J. Virol. Methods 48: 211-221, 1994. PubMed: 7989438**
27802: Vassilev VB, et al. Authentic and chimeric full-length genomic cDNA clones of bovine viral diarrhea virus that yield infectious transcripts. J. Virol. 71: 471-478, 1997. PubMed: 8985373

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Cell Biology

ATCC® Number: **CRL-2843™** Order this Item Price: **\$338.00**
 Designations: 3D4/21 Depositors: J Gren
Biosafety Level: 2 [cells containing SV40 viral DNA sequences] Shipped: frozen
 Medium & Serum: See Propagation Growth Properties: adherent
 Organism: Sus scrofa (pig) Morphology: macrophage

Source: **Organ:** lung
Strain: Landrace
Cell Type: macrophage macrophage (alveolar); immortalized with SV40 large T antigen transformed with pSV3-neo

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Isolation: **Isolation date:** December, 1998
 Bovine adenovirus 3
 Classical swine fever virus
 Human parainfluenza virus 3
 Swinepox virus
 Vesicular stomatitis New Jersey virus

Virus Susceptibility: Porcine adenovirus
 Herpes simplex virus 1
 African swine fever virus
 Pseudorabies virus
 Vaccinia virus
 Swine vesicular disease virus

Age: 27 days

Gender: unknown

Comments: The parental porcine monomyeloid cell line, 3D4, was established in December of 1998 following transfection of primary porcine alveolar macrophage cultures with the pSV3neo plasmid. The plasmid carries the genes for neomycin resistance and SV40 large T antigen. Single cell cloning and selection in G-418 of the 3D4 parental cell line resulted in establishment of 3D4/2 (ATCC CRL-2845), 3D4/21 (ATCC CRL-2843) and 3D4/31 (ATCC CRL-2844). A subpopulation of each cell line was positive, to varying degrees depending on the media formulation, for nonspecific esterase activity and phagocytosis. Clone 3D4/21 can produce Bovine adenovirus type 3 (BAV-3) to markedly higher titers than clones 3D4/2 and 3D4/31. Addition of DMSO improved the capability of clone 3D4/21 to replicate the field isolate of African swine fever virus (ASFV/Lillie) compared to the other clones. These porcine myelomonocytic cell lines may have a wide variety of applications in porcine virology and immunology [PubMed:12088830].

Propagation: **ATCC complete growth medium:** RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate supplemented with 0.1 mM nonessential amino acids, 90%; fetal bovine serum, 10%

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

Protocol: Volumes used in this protocol are for 75cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

Subculturing:

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 2008
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 are 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. This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

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

Completed forms are to be returned to Occupational Health and Safety, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR C. Yong Kang
SIGNATURE CYKang
DEPARTMENT Microbiology and Immunology
ADDRESS 1460 Western Road, Rm 129, SDRI, London, ON, N6G2V4
PHONE NUMBER 519-661-3226
EMAIL CYKang@UWO.ca

Location of experimental work to be carried out: Building(s) SDRI Room(s) 129, 124

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: Sumagen Canada Inc
GRANT TITLE(S): Development of Hepatitis C Virus Vaccine

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

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

Gyoung Nyoung Kim
Hwayong An
Kunyu Wu
Chad Michalski
Elizabeth Banaszkowska
Raji Singh

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
<i>Vesicular Stomatitis Virus (VSV) (Adapted)</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	0.1 L		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 3
<i>Adenovirus</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	2 L		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	A549, HepG2	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Baby hamster kidney Chinese hamster ovary	ATCC
Non-human primate	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Vero	ATCC
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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 HC or CFIA containment level required 1 2 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO

If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α XL10 Gold	Transcription Vector (pTV)	Dr. Andrew Ball University of Texas	VSV genome and HCV genes	HCV genes were inserted into the VSV genome at S gene and L gene junction

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

4.3 Will genetic modification(s) involving viral vectors be done? YES, complete table below NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
Vesicular Stomatitis Virus (Lab. adapted)	Indiana & New Jersey serotypes	Lab. adapted virus - Dr. Kang Lab.	HCV genes	HCV genes are inserted into VSV genome.
Adeno Virus	Serotype 5	Microbix biosystem	HIV Gag gene	HIV Gag gene replaces Ad5 E1 gene

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? YES NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? YES NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used mouse

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals YES, specify: mouse NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

9.0 Import Requirements

9.1 Will the agent be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 10.0

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

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

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

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

10.0 Training Requirements for Personnel Named on Form

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

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS
- ◆ 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 *C. Kang* x

11.0 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. ○ 1 2 ○ 3

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

- YES, permit # if on-campus _____
- NO
- NOT REQUIRED

12.0 Procedures to be Followed

12.1 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. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE *C. Kang* Date: June 16, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: *S.M. Kelder*
Date: 4 Nov. 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: *J. Stanley*
Date: Oct 31/08

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

Approval Number: BIO-UWO-0004 Expiry Date (3 years from Approval): Nov. 04, 2011

Special Conditions of Approval: Dr. Kang:

Contact Dr. J. Bell @ Ottawa Hospital Research Institute
613-737-7700 x70333 to ensure similar protocols in place.