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

Raji Singh

Gyoung Kim

Additional Personnel
(Please list additional personnel here)

	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. Coli (DH 5 alpha, xL10 Gold)	
Approved Primary and Established Cells	Human (established): A549, Hep G2. Rodent (established): Baby hamster kidney, Chinese hamster ovary. Non-human Primate (established): Vero, 3D4/21- Pig alveolar Mo, EBTr - Bovine trachea cell, E. Derm-horse	APC49 (Huh 7.5) Human established HCN-1A (Human Cortical Neuron) from ATCC
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	Transcription vector (pTV), HCV genes inserted into VSV genome, Adeno virus serotype 5 replaced by HIV Gag gene	APP241 (H1SG-Neo) NS3 to NS5b HCV genes (non structure proteins) in pBR322 vector
Approved Use of Animals	Mouse	
Approved Biological Toxin(s)		

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

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

Signature of Permit Holder: E G Kong

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

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

Date of Last Modification (if applicable): Apr 24, 2009

BioSafety Officer(s):

Chair, Biohazards Subcommittee: **Date:**

-------- Original Message --------
Subject: Re: Technical information such as MSDS request - from UWO Safety Dept.
Date: Wed, 07 Jul 2010 09:19:46 -0500
From: Robert Roth <roth@apath.com>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer,
Let's start with some background information. All materials from Apath are received via an Academic Material Transfer Agreement. Transfers within an institution is not permitted. All materials are specifically provided to an independent researcher. The two items you've listed are utilized under BSL2 standards and are for research use only. We do not have a MSDS generated for these materials. Additional information specific to these constructs is available via Dr. Kang with respect to information provided in the scientific literature. Please have Dr. Kang contact Randi Cowperthwiate to begin the MTA process.
Kind Regards,
Bob

Robert M. Roth, M.S., MCSA, MCDBA | Vice President | Strategic Operations
Please send all hard copy correspondence c/o Randi Cowperthwiate
Apath,LLC | 760 Parkside Avenue | Suite 310
Brooklyn, NY 11226-1508 | USA
Phone: (347) 533-4834 ext. 102 | Fax: (347) 533-4835
E-mail: roth@apath.com

A brief description of HCN-1A and the work that requires the cell line

HCN-1A is a human cortical neuronal cell line. We will use the cell line for the infectivity studies of Vesicular stomatitis virus.

HCN-1A belongs to the BSL 1

A brief description of Huh-7.5 and the work that requires the cell line

Huh-7.5 is a human hepatoma cell line, Huh-7 subline. We will use the cell line for the replication of Hepatitis C virus (HCV) subgenomic replicon.

Huh-7.5 belongs to the BSL 1

H/SG-Neo (pHCVrep13/Neo) is a vector containing the HCV 5' NTR, the neomycin phosphotransferase gene (Neo), which upon expression confers resistance to G418, the IRES from encephalomyocarditis virus (EMCV), which directs translation of HCV proteins NS3 to NS5B, and the 3' NTR. We will use the vector as positive control.

Cell Biology

ATCC® Number:	**CRL-10442™** Order this Item	Price:	**$324.00**
Designations:	HCN-1A		
Depositors:	Johns Hopkins University		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	neuronal 		
Source:	**Organ:** brain **Cell Type:** cortical neuron;		
Cellular Products:	tubulin; neurofilament protein; somatostatin; cholecystokinin-8		
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.		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11,12 D16S539: 12 D5S818: 11,12 D7S820: 11,12 THO1: 9.3 TPOX: 11 vWA: 17		
Age:	18 months		
Gender:	female		
Comments:	The cells stain positively for a number of neuronal markers including neurofilament protein, neuron specific enolase (NSE). [48286] They are also positive for tubulin, vimentin, somatostatin (SST), glutamate, gamma aminobutyric acid (GABA), cholecystokinin - 8 (CCK-8) and vasoactive intestinal peptide (VIP). [22022] The cells are negative for glial fibrillary acidic protein (GFAP) and myelin basis protein (MBP). [48286] HCN-1A cells can be induced to differentiate when cultured with a mixture of nerve growth factor (NGF), dibutyryl cyclic adenosine monophosphate (cAMP) and 1-isobutyl-3-methylxanthine (IBMX). [22022] Differentiation is accompanied by mature morphology and slowing of growth (doubling time greater than 120 hours). [22022] Unlike HCN-2 (see ATCCCRL-10742) the growth rate of HCN-1A cells is not affected by phorbol esters. [22022]		

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required ▸

Product Information Sheet

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

Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Growth Conditions:** The growth medium must be adjusted to pH 7.35 prior to filtration **Protocol:**
Subculturing:	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 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes. 6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. 7. Place culture vessels in incubators at 37C.
Preservation:	CRL-10442 has been shown to senesce at approximately passage 19. Current distribution stocks are prepared with a minimum of only 2 passages remaining under recommended culture conditions after cryopreservation. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended **Medium Renewal:** 1 to 2 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	22022: Ronnett GV, et al. Human neuronal cell line. US Patent 5,196,315 dated Mar 23 1993 48286: Ronnett GV, et al. Human cortical neuronal cell line: establishment from a patient with unilateral megalencephaly. Science 248: 603-605, 1990. PubMed: 1692158

Return to Top

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Permit Holder: Chil Kang

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

Hwayong An

Kunyu Wu

Elizabeth Banaskowska

~~Chad Michalski~~

Raji 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. Coli (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 MΦ EBTr - 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: C J Kang

BioSafety Officer(s): J Stanley April 24/09

Chair,Biohazards Subcommittee: Glen Kidder

Modification Form for Permit BIO-UWO-0064

Permit Holder: Chil Kang

Approved Toxin(s)

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
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Classification: 2

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

Signature of Permit Holder: C Kang

BioSafety Officer(s): Stanley April 24/09

Chair,Biohazards Subcommittee: C Kidder

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.

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 C.Y.Kang
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:

GyoungNyoun Kim
Hwayong Ar
Kunyu Wu
*Chad Michalski
Elizabeth Banasikowska
Raji Singh

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

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
Vesicular Stomatitis Virus (Lab adapted)	○ Yes ☒ No	☒ Yes ○ No	☒ Yes ○ No	0,1 L		○ 1 ☒ 2 ○ 3
Adenovirus	☒ Yes ○ No	○ Yes ☒ No	○ Yes ☒ No	2 L		○ 1 ☒ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 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	○ Yes ☒ No		Not applicable
Rodent	○ Yes ☒ No		
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ 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	☒ Yes ○ No	A549, HepG2	ATCC
Rodent	☒ Yes ○ No	baby hamster Kidney Chinese hamster ovary	ATCC
Non-human primate	☒ Yes ○ No	Vero	ATCC
Other (specify)	○ Yes ☒ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier (For more information, see www.atcc.org) ○

2.4 For above named cell types(s) indicate 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? O YES ⊗ NO
If no, please proceed to Section 4.0

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material 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		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 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 O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ⊗ YES, complete table below O 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 G 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 O 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 O YES, please specify _____ ⊗ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ⊗ NO
- SV 40 Large T antigen O YES ⊗ NO
- E1A oncogene O YES ⊗ NO
- Known oncogenes O YES, please specify _____ ⊗ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ⊗ NO

4.5 Will virus be replication defective? O YES ⊗ NO

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

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used mouse

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals ☒ YES, specify: mouse O 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 O YES ☒ NO
- Pound source cats O YES ☒ NO
- Cattle, sheep or goats O YES ☒ NO
- Non- Human Primates O YES, please specify species _____ ☒ NO
- Wild caught animals O YES, please specify species & colony # _____ ☒ NO
- Birds O YES ☒ NO
- Others (wild or domestic) O YES, please specify _____ ☒ NO

8.0 Biological Toxins

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

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

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

9.0 Import Requirements

9.1 Will the agent be imported? O 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? O YES O NO

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

9.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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.Y. 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. O 1 ☒ 2 O 3

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

☒ YES, permit # if on-campus_____
O NO
O 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 x C.Y. Kang Date: June 16, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: S.M. K. Lee
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-00064 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 simular protocols in place.

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