Modification Form for Permit BIO-RRI-0033

Permit Holder: Michael Rieder

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

Evan Russel

Laura Cheng

Anda Marcu *

Abdelbaset Elzagallaai *

Lauren Hanly

Additional Personnel
(Please list additional personnel here)

* These two people will be working on this project.

	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	Clostridium difficile, E. coli DH10BTonA	Heat Killed Listeria monocytogenes
Approved Primary and Established Cells	Human [primary]: PBLs. Human [established]: Jurkat E 6.1; Human cardiomyocytes:HK-2, HepG2.	
Approved Use of Human Source Material	Human Blood(whole) or other body fluid: Patients with adverse drug reactions.	
Approved Genetic Modifications (Plasmids/Vectors)	[plasmids]: pJ3omega-MDR3 SLC28A3_Human. [oncogenes]: HPV-16, E6/E7.	
Approved Use of Animals		
Approved Biological Toxin(s)		

* *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..*

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: Apr 14, 2011

Date of Last Modification (if applicable): _____

BioSafety Officer(s): Ronald Hozempa Sept.06,2011

Chair, Biohazards Subcommittee: _____ **Date:** _____

- Heat killed work only can be done at Robarts Rooms - 2226/2220 (Level 2).

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.

Heat killed Listeria Monocytogenes (LM) bacteria is a heat treated non-viable bacteria. The purpose of obtaining heat killed LM is to be used as a substitute to live bacteria in experiments that require total LM antigens. Using heat killed LM is safer as the material is no longer infective and carry no biological hazard as the live bacteria.

Heat Killed *Listeria monocytogenes* will be handled under biosafety level 2 (according to PHAC) and the personnel informed about the hazard (Material Safety Data Sheet).

- use of biosafety cabinets for activities generating aerosols; laboratory coat, gloves and eye protection worn

- stored in sealed containers that are appropriately labeled;

- in case of accidental spills: allow aerosols to settle, wear protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite starting at the perimeter and working towards the centre, allow sufficient contact time (30 min) before clean up.

- disposal: all materials autoclaved; used glassware and other supplies in contact with infectious materials are to be place in sturdy, heat resistant container and autoclaved; disposable materials (gloves, tissue paper, etc) are collected as biohazardous waste and autoclaved

Dr. Uma Mahesh Babu – project co-leader (Hygea Life Sciences liaison)
Expertise – development of rapid diagnostic kits

Dr. K. Adeyanju – post-doctoral fellow
Expertise – immunology

Dr. Abdelbaset Ezagallaai – post-doctoral fellow
Expertise – *in vitro* diagnostics

Project description

Objective: Develop a "Proof of Principle" Model (POP) which demonstrates the contemporaneous testing of a single liquid sample for the presence of multiple marker proteins, using a single test device. A qualitative (yes/no) visual test to determine positive or negative (control spot will show) results within 2 minutes.

The design for the "POP model" is to develop a 2 spot *Listeria monocytogénes* test and to complete lab development with gold standards, within an 8 month window. The field clinical trials would follow and be completed in a further 2-3 months.

Test Action: A liquid sample (from a swab or a wash) is applied to the test area and through gravitational force and surface tension, moves down to the protein marker layer embedded in the test structure. Thereafter a drop of a selected reagent is applied so that it covers the visible portion of the active surface area and its role is to operatively bind to any marker protein that may have been immobilized.

Specificity is conferred by the selection of appropriate antibodies. The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110-130 amino acids, give the antibody its specificity for binding a specific antigen, in this case to *Listeria*. (Initial phase of the project will involve working with heat killed *L. monocytogenes*.)

This is an **antigen** detection test. It has the potential to test other species other than *Listeria*. In order to detect an antigen in the sample, a pair of highly specific antibodies are needed. One part of the pair is immobilized in the Test area as a spot. The other part of the pair is chemically attached to a visual nanopartical called conjugate. The antigen is literally sandwiched between these two parts in the pair and gives rise to a visually detectable spot. Developing the 'harmony' of various antigens within the same protein layer to respond to the same sample, at the same time, requires extensive analysis and lab experimentation to achieve the desired functionality.

Timeline

The test's evolution will be designed to use the following **2 phase** timing model:

The **first phase** will incrementally add the desired first part of the antibody pair for *Listeia* into one combination spot. If this is, then the spot will activate. If none are

InvivoGen

Material Safety Data Sheets

Section 1 - Product and Company Information

Product name: **HKLM**

Cat. code: tlrl-hklm

Company identification:
InvivoGen, 3950 Sorrento Valley Blvd, Suite 100
San Diego, California 92121, USA
(+1) 858 457 5873

Cayla-InvivoGen, 5 rue Jean Rodier
31400 Toulouse, FRANCE
+33 (0) 5 62 71 69 39

Emergency number: (+1) 888 457 5873 (Monday - Friday, 8.00 am – 6.00 pm)

Disclaimer: All InvivoGen products are supplied for research and laboratory use only. Not for drug, household or other uses.

Section 2 - Hazards Identification

Emergency Overview
OSHA Hazards No known OSHA hazards.

Not a dangerous substance according to GHS.

GHS Label elements, including precautionary statements

Pictogram	None
Signal word	None
Hazard statement	None
Precautionary statements	None

HMIS Classification Health Hazard **0** Flammability Hazard **0** Reactivity Hazard **0**
NFPA Rating Health Hazard **0** Fire **0** Reactivity Hazard **0**

Potential Health Effects
- Eye contact: May cause eye irritation.
- Skin contact: May cause skin irritation.
- Skin absorption: May be harmful if absorbed through the skin.
- Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
- Ingestion: May be harmful if swallowed.

Section 3 – Composition/Information on Ingredient

Substance Name: Heat killed *Listeria monocytogenes*
CAS Number: Not available

Section 4 – First Aid Measures

General advice: Consult a physician. Show this material safety data sheet to the doctor in attendance.
After skin contact: Immediately wash skin with soap and plenty of water. Consult a physician.
After swallowing: Never give anything by mouth to an unconscious person. Rinse mouth with water provided person is conscious. Consult a physician.
After inhalation: Remove to fresh air. If not breathing give artificial respiration. Consult a physician.
After eye contact: Immediately flush eyes with plenty of water for at least 15 minutes. Consult a physician.

Section 5 – Fire Fighting Measures

Suitable extinguishing media: Water spray, carbon dioxide, dry chemical powder or appropriate foam.
Special Firefighting Procedures: Wear self-contained breathing apparatus for fire fighting if necessary.

Section 6 – Accidental Release Measures

Personal precautions:
Wear protective equipment. Keep unprotected persons away. Avoid dust formation.
Method for Cleaning Up:
Sweep up and place in closed containers for disposal. Dispose contaminated material as waste according to section 13. Ventilate area and wash spill site after material clean-up is complete.

Section 7– Handling and Storage

Handling: Avoid contact with eyes, skin and clothing. Avoid prolonged or repeated exposure.
User exposure: Avoid Inhalation. Use personal protective equipment (i.e. impermeable gloves, lab coat or apron).
Storage: Store at 4°C.

Section 8 – Exposure Controls / PPE

Engineering measures: Ensure adequate ventilation, especially in confined areas.

Personal Protective Equipment
Hand: Protective gloves to prevent skin contact. **Eye:** Chemical safety goggles
General hygiene measures: Wash hands thoroughly after handling.

Section 9 – Physical / Chemical Properties

Appearance: Light brown color
Physical state: Solid (lyophilized cells)

Section 10 – Stability and Reactivity

Stability: Stable
Hazardous polymerization: Will not occur
Materials to avoid: Strong oxidizing agents
Hazardous decomposition products: Nature of decomposition products are not hazardous.

Section 11 – Toxicological Information

Acute toxicity: No data available.
Skin irritation/corrosion: No data available.
Serious eye damage/eye irritation: No data available.
Respiratory or skin sensitization: No data available.
Additional toxicological information: No data available.

Section 12 – Ecological Information

Ecotoxicity: No data available.
Persistence and degradability: No data available.
Bioaccumulative potential: No data available.
Mobility in soil: No data available.

Section 13 – Disposal Considerations

Product:
Observe all federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Must not be disposed of together with household garbage.
Contaminated packaging:
Dispose of as unused product.

Section 14 – Transport Information

DOT (US): Not dangerous goods
IATA: Not dangerous goods
IMDG: Not dangerous goods

Section 15 – Regulatory Information

OSHA HAZARDS (US) No known OSHA Hazards
DSL Status: This product contains the following components that are not on the Canadian DSL nor NDSL lists.
Heat killed *Listeria monocytogenes* CAS number: not available
SARA 302 Component: None of the ingredients are listed.
SARA 313 Component: None of the ingredients are listed.
SARA 311/312 Hazards: None of the ingredients are listed.

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

Labeling and risk phrase according to EU Directives
The product does not need to be labeled in accordance with EC directives or respective national laws.

Section 16 – Other Information

The information contained in this MSDS relates only to the material(s) designated and does not relate to use(s) in combination with any other material, process(es) and/or chemical reaction(s). InvivoGen provides this information in good faith and is based on our present knowledge. This MSDS is provided without warranty of any kind. The recipient is responsible for ensuring that, where applicable, existing laws and guidelines are observed.

Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7088 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7088 Télec: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

Laboratory Compliance to *Containment Standards for Veterinary Facilities*

The Office of Biohazard Containment and Safety (OBCS) has received and reviewed the Inspection Checklist for the Animal Pathogen Containment Level 2 Facility below. This letter serves to confirm the OBCS has found the information provided to be **acceptable for work *in vitro***.

Organization:	The University of Western Ontario Robarts Research Institute
Address:	100 Perth Drive London, Ontario N6A 5K8
Attention:	Anda Marcu, Dr. Michael J. Rieder and Ron Noseworthy
Phone Number:	519-931-5777
Laboratories:	Rooms 2226 and 2220
CFIA Compliance Number:	C-2010-0033-4
Compliance Letter expiry date:	January 14, 2012

For your reference, the *Containment Standards for Veterinary Facilities*, from which the inspection checklist was adapted, are available on the internet at the following address: http://www.inspection.gc.ca/english/sci/bio/bioe.shtml. Please visit our website for more information and updates on our program.

Note: Canadian distributors of biological products (animal pathogens) regulated under the *Health of Animals Act* will require their clients to submit a copy of this letter.

Please do not hesitate to contact the Office of Biohazard Containment and Safety of the CFIA if you have any questions regarding this letter.

Sincerely,

Cinthia Labrie

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

20 JAN. 2010
Date

Canada

Public Health Agency of Canada | Agence de la santé publique du Canada

Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3rd Ed., 2004*

This letter serves to confirm that the Office of Laboratory Security has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

Organization:	University of Western Ontario Robarts Research Institute
Attention:	Dr. Michael Rieder
Address:	100 Perth Drive London, ON N6A 5K8
Laboratory Room Number(s):	2220
Type of work:	■ *in vitro* only ☐ *in vitro* and *in vivo**
Compliance Letter expiry date:	July 28, 2011.

To renew your compliance letter please complete a CL2 checklist and fax it to our office at (613) 941-0596. The checklist can be obtained from the following website: www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz
Chief, Importation and Regulatory Affairs

SEPTEMBER 23, 2009
Date

*The Office of Laboratory Security must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses.

Canada

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. Michael Rieder
DEPARTMENT	Biotherapeutics / Paediatrics
ADDRESS	100 Perth Drive, RRI, Room 2226
PHONE NUMBER	519 931 5777 x 24209
EMERGENCY PHONE NUMBER(S)	519 931 5777 x 24209
EMAIL	mrieder@uwo.ca

Location of experimental work to be carried out: Building(s) ___Robarts_____ Room(s)_2220, 2226___

*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:_____CIHR, CIHR-GSK, CIHR-CFI_____
GRANT TITLE(S): _____Vincristine Neurotoxicity
_____Canadian Pharmacogenomic Network for Drug Safety
_____

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

Name	UWO E-mail Address	Date of Biosafety Training
Anda Marcu	amarcu2@uwo.ca	19-July-2006
Lauren Hanly	lhanly@uwo.ca	1- Oct.- 2008
Laura Cheng	lcheng2@uwo.ca	1-Oct.-2008
Abdelbaset Elzagallai	aelzagal@uwo.ca	14-May-2007
Evan Russel	erussel5@uwo.ca	22-Jan.-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.

The human hepatocellular liver carcinoma (HepG2) cell line is being used to look at the effects of increasing concentrations of the antiepileptic drug, valproic acid (VPA). In addition we would like to look at the effects when these cells are pretreated with VPA and supplemented with increasing concentrations of folic acid to see if folic acid can reverse the negative effects of VPA.
The HepG2 cells are stored and cultured according to the instructions on the attached product sheet.

HK-2 cells are cultured and treated with the chemotherapy agent Ifosfamide, as well as several antioxidants in order to assess the nephrotoxic effects of Ifosfamide and the ability of antioxidants to attenuate it. These cells are stored and cultured according to the instructions on the attached product sheet.

Human Cardiomyocyte cells (HCM) are used for in vitro assessment of Anthracyclines toxicity. The cells are to be transfected using ABCB4 and SLC28A transporters plasmid DNA in order to assess the Anthracyclines toxicity on the transfected HMC.
HCM cells are stored and cultured according to the instructions on the attached product sheet.
*None of the drugs mentioned above are controlled substances

Jurkat cells and the human peripheral blood cells (isolated from the blood from patients with adverse drug reactions) are both used in in vitro experiments to asses drug toxicity. Both types of cells are incubated with drugs at specific concentrations.

Waste is treated as biohazard material and decontaminated before disposal. (cells are bleached; flasks, containers & tubes are autoclaved)

Please include a one page research summary or teaching protocol.

Research is directed to understanding how adverse drug reactions develop, how to predict the risk for adverse drug reactions and how to design safer and more effective drugs. His particular focus is on adverse drug reactions mediated by reactive drug metabolites. Many commonly used and important drugs are metabolized, at least in part, to reactive intermediates. Drug toxicity is assessed by in vitro experiments, the metabolism studies consist of incubating drugs at specific concentrations with human peripheral blood cells, or other cell lines, with or without rat or human liver microsomes. The viability of cells is determined with and without microsomes present, this giving the indication of the toxicity of the drug for the patient or cell line in the presence of a drug metabolizing agent.

Please include a one page research summary or teaching protocol.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? OX YES O xNO
If YES, please give the name of the species. Clostridium difficile
What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated: **SPILLS:** Allow aerosols to settle; wear protective clothing; gently cover spill with paper towels and apply a suitable disinfectant (high level or 1% sodium hypochlorite), starting at perimeter and working towards the centre; allow sufficient contact time before clean up. **DISPOSAL:** Decontaminate before disposal; steam sterilization, chemical disinfection, incineration. **STORAGE:** In sealed containers that are appropriately labeled

Please attach the CFIA permit.

1.2 Please complete the table below: C.diff. work & culture will be done at Victoria Hospital

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
C. difficile (Clostridium)	☐X Yes ☐ No	☐X Yes ☐ No	☐ Yes ☐ XNo	- * 10-15mL	ATCC	☐ 1 ☐X 2 ☐ 2+ ☐ 3
E. coli DH10B TonA	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☐ No	* *		☐ 1 ☐ 2 ☐ 2+ ☐ 3 (host strain DH10B TonA)
	☐ Yes ☐ No	☐ Yes ☐ No	See e-mail			☐ 2 ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No				☐ 2 ☐ 3

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

* Not culturing any C. diff at the moment. We have ~0.5ml freeze dried.
** Not culturing any E. coli at the moment.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐X 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐X Yes ☐ No	~~ATCC, ScienCell~~ PBL) - Human Volunteers	Not applicable
Rodent	☐ Yes ☐X No		
Non-human primate	☐ Yes ☐X No		
Other (specify)	☐ Yes ☐X No		

Subject: Biological Agents Registry Form: Reider
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 12 Apr 2011 11:44:53 -0400
To: Gail Ryder <Gail.Ryder@LawsonResearch.Com>

Gail
I wanted to let you know about this - the Rieder lab is proposing to do a project involving C. difficile at Victoria Hospital.
Regards
Jennifer

-------- Original Message --------
Subject: Re: FW: Biological Agents Registry Form: Reider
Date: Fri, 01 Apr 2011 12:19:15 -0500
From: Anda Marcu <amarcu2@uwo.ca>
To: Ron Noseworthy <rnoseworthy@robarts.ca>, jstanle2@uwo.ca

Re: FW: Biological Agents Registry Form: Reider

Clostridium difficile full name should be listed in section one - noted.

We haven't started working on this project, so we are not culturing any C. difficile at the moment. Once we start culturing C. difficile, I'm assuming it will be ~ 10-15 mL at a time.

The off campus location: Victoria Hospital, Room E3-206.

Thank you!

E-mail

Anda Marcu

April 12, 2011 - confirmed w Anda, no enterohemorrhagic E.coli used.

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 ☐ No	Jurkat E 6.1; Human Cardiomyocytes; HK-2, HepG2	ATCC, ScienCell
Rodent	☐ Yes ☐X No		
Non-human primate	☐ Yes ☐X No		
Other (specify)	☐ Yes ☐X No		

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

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☐ 1 ☐X 2 ☐ 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 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	Patients with Adverse Drug Reactions	☐ Yes ☐X Unknown		O 1 OX 2 ☐ 2+ O 3
Human Blood (fraction) or other Body Fluid	N/A	☐ Yes ☐ Unknown		O 1 O 2 ☐ 2+ O 3
Human Organs or Tissues (unpreserved)	N/A	☐ Yes ☐ Unknown		O 1 O 2 ☐ 2+ O 3
Human Organs or Tissues (preserved)	N/A	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 ☐ 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 from transformation or tranfection
E.coli DH 10B TonA *E.coli*	*pJ3omega-MDR3 SLC28A3_Human*	ATCC	ABCB4 gene SLC28A3	*.transfected Human Cardiomyocytes*

host strain DH 10B TonA

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

Add a transgene (ABCB4)

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV ☐ 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 HPV-16 E6/E7 ☐ 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 involving a biological agent? ☐ YES ☐ XNO
(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? ☐ YES ☐ 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? ☐ YES, number: _____ ☐ NO ☐ PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ☐ 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 ☐ YES, specify: _____ ☐ NO

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

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ☐ YES ☐ XNO 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? ☐ YES, Please provide details:_____ ☐ 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? ☐ 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? ☐ 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? ☐ YES ☐ NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? ☐ 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? ☐ Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? ☐ YES ☐ 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? ☐ YES ☐ 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? X YES, please give country of origin U.S. ATCC ☐ NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? X YES, please provide permit # C-2010-0033-4 ☐ 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. ☐ 1 ☐ X2 ☐ 2+ ☐ 3

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

☒ YES, permit # if on-campus: BIO-RRI-003
☐ NO, please certify
☐ 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: Dec 20 2010

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.

We are following containment level 2 biosafety guidelines.

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

Squeeze the area surrounding the needle stick injury to expel blood; wash the wound with cold running water; apply antiseptic & band-aid; contact health services.

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 14 April 2011

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: April 12, 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: January 31, 2011

Approval Number: BIO-RRI-0033 Expiry Date (3 years from Approval): April 13, 2014

Special Conditions of Approval:

- Discuss work at Victoria Hospital involving C. difficile with Gail Ryder (LHRI safety officer) before it starts.

Clone

ATCC® Number:	**65706** Order this Item	Price:	**$167.00**

Designation:	pJ3omega-MDR3 [3.27]
Depositors:	P Borst
Other Id's:	GenBank:M23234
Insert Source:	*Homo sapiens*
Insert Information:	DNA: cDNA Insert lengths(kb): 4.0 Tissue: liver Gene product: P glycoprotein 3/multiple drug resistance 3 [MDR3] Target Gene: P glycoprotein 3/multiple drug resistance 3
Biosafety Level:	1
Shipped:	freeze-dried
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:	in another host, produces protein P glycoprotein 3/multiple drug resistance 3 [27354]
Vector:	Size (kb): 3.5000000000000000 Vector: pJ3omega (plasmid) Promoters: Promoter SV40 early Construction: pBR322, SV40 Marker(s):ampR Construct size (kb): 3.5 Features: marker(s): ampR other: SV40 t IVS promoter: SV40 early replicon: SV40 replicon: pMB1 MCS: HindIII...BglII terminator: SV40

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Media and Antibiotics

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:	Restriction digests of the clone give the following sizes (kb): HindIII/XbaI--4.0, 3.5; BamHI--4.5, 3.0; AvaI--4.6, 2.0, 0.5, 0.4; EcoRI--3.6, 2.8, 1.2; PstI--2.9, 2.3, 0.9, 0.6, 0.5, 0.4. The insert contains the following restriction sites (nt positions from the 5' end): BamHI--987; XhoI--1246; EcoRI--2816. [27333] A full-length cDNA clone extending from nt -33 to +4002 relative to the translation initiation codon. The coding sequence is 3839 nt. [27333] When transfected into BRO melanoma cells, the sequence expresses a protein recognized by monoclonal antibodies but does not confer multiple drug resistance. [27354] Originally cloned using EcoRI linkers into the polylinker of lambdaZAP. Excised as a HindIII/XbaI fragment (sites from the polylinker) for insertion into pJ3omega. [115337]
Media Description:	ATCC medium 1227: LB Medium (ATCC medium 1065) with 50 mcg/ml ampicillin
References:	27333: van der Bliek AM, et al. Sequence of mdr3 cDNA encoding a human P-glycoprotein. Gene 71: 401-411, 1988. PubMed: 2906314 27354: Schinkel AH, et al. Characterization of the human MDR3 P-glycoprotein and its recognition by P-glycoprotein-specific monoclonal antibodies. Cancer Res. 51: 2628-2635, 1991. PubMed: 1673638 115337: Piet Borst, personal communication

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Product Page	(click to open) - (see related products)
Catalog Number	MHS1010-98052382
Clone ID	7939668 Clone Details
Description	Human MGC Verified FL cDNA (IRAT)
Detailed Description	NIH_MGC_294
Accessions	BC093823, BC093823.1
Host Strain	DH10B TonA
Tissue	colon
Species	Homo sapiens
Location	118-e -4
Comment	Inserts are flanked by and can be excised using EcoRI as long as inserts do not contain any internal EcoRI sites
3' Restriction Site	TA cloning
5' Restriction Site	TA cloning
Vector Name	pCR4-TOPO
Vector Type	Non Expression
Antibiotic Information	Ampicillin (Concentration: 100 µg/ml, Resistant Range: 100-100 µg/ml) Kanamycin (Concentration: 25 µg/ml, Resistant Range: 25-25 µg/ml)
Sequencing Primers	M13 (-21), M13 reverse, T7, T3

Product Page	(click to open) - (see related products)
Catalog Number	MHS1010-98052357
Clone ID	7939666 Clone Details
Description	Human MGC Verified FL cDNA (IRAT)
Detailed Description	NIH_MGC_294
Accessions	BC093821, BC093821.1
Host Strain	DH10B TonA
Tissue	colon
Species	Homo sapiens
Location	118-d -1
Comment	Inserts are flanked by and can be excised using EcoRI as long as inserts do not contain any internal EcoRI sites
3' Restriction Site	TA cloning
5' Restriction Site	TA cloning
Vector Name	pCR4-TOPO
Vector Type	Non Expression
Antibiotic Information	Ampicillin (Concentration: 100 µg/ml, Resistant Range: 100-100 µg/ml) Kanamycin (Concentration: 25 µg/ml, Resistant Range: 25-25 µg/ml)
Sequencing Primers	M13 (-21), M13 reverse, T7, T3

Technical Manual

Thermo Scientific Open Biosystems cDNA Clones and Plates

Product Description

Clones are provided as *E. coli* cultures in LB broth with 8% glycerol, an inert growth indicator, and the appropriate antibiotic at the concentration indicated in Table 1.

Table 1. Cap Color Code

Antibiotic	Concentration	Utility
Red	Ampicillin	100 µg/ml
Black	Chloramphenicol	25 µg/ml
Green	Kanamycin	25 µg/ml

Shipping And Storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4°C. They may stored indefinitely at –80°C.

Plates are shipped on dry ice and should be stored at –80°C.

Clone Verification

For cDNA clones and other genomic resources, there is a small possibility of mistaken identification, incorrect DNA sequence, or incorrect annotation. In cases of mistaken identification, we will supply the correct clone if possible.

All DNA sequences and annotations have been submitted to GenBank by the supplier (for example, the IMAGE consortium), but have not been independently verified by Thermo Scientific Open Biosystems. We therefore strongly recommend the following routine precautions:

1. Prior to purchase, the customer should analyze the database sequence for the clone of interest using BLAST or other bioinformatics tools.
2. After purchase, the customer should end-sequence the clone and BLAST the result against the GenBank sequence.

Getting Clone Information

The Thermo Scientific Open Biosystems Gene Query provides a rapid means of locating relevant clone information. Simply enter a clone ID number or accession number into the query box and click "submit" (Figure 1 – yellow arrow).

Clicking the appropriate link on the query result page for your type of clone will display the clone information page (Figure 1 – pink arrow).

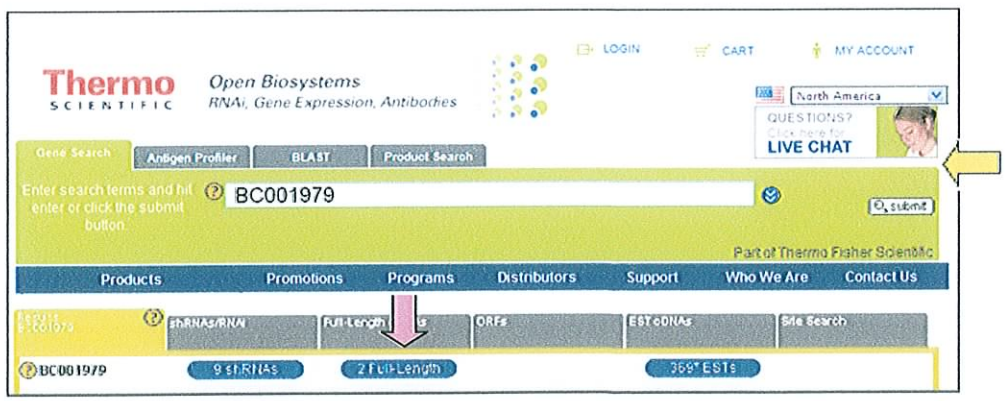

Figure 1. Thermo Scientific Open Biosystems Gene Query and Gene Query Results

Clicking on the link under 'click for details' will open a window at the bottom of the page where pertinent information such as cloning details, sequence information, etc can be found (Figure 2).

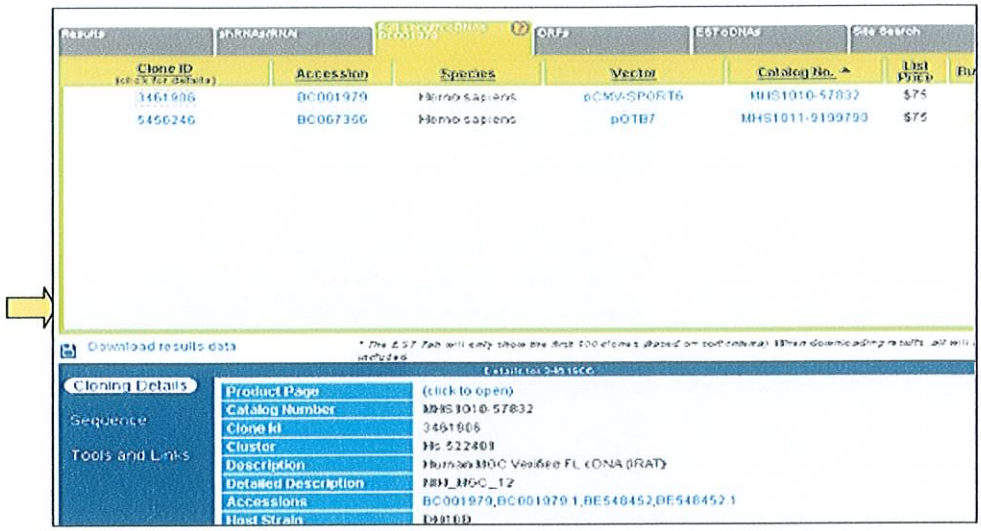

Figure 2. Thermo Scientific Open Biosystems Gene Query Details

Verifying Individual cDNA Clone Identity

We recommend picking at least 5 independent colonies for verification to ensure that the clone of interest is derived from a single isolate.

By Sequencing

We further recommend verification of clones by end sequencing. The sequencing primers appropriate for each vector can be obtained from Details > View screen shown in Figure 2. A useful tool for comparing the sequence obtained to the sequence expected is to perform a pairwise BLAST. The link to this feature on the NCBI website is: http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi.

Simply enter the sequence you obtained in the Sequence 1 window and enter the sequence retrieved from the Clone Details screen in the Sequence 2 window (Figure 3).

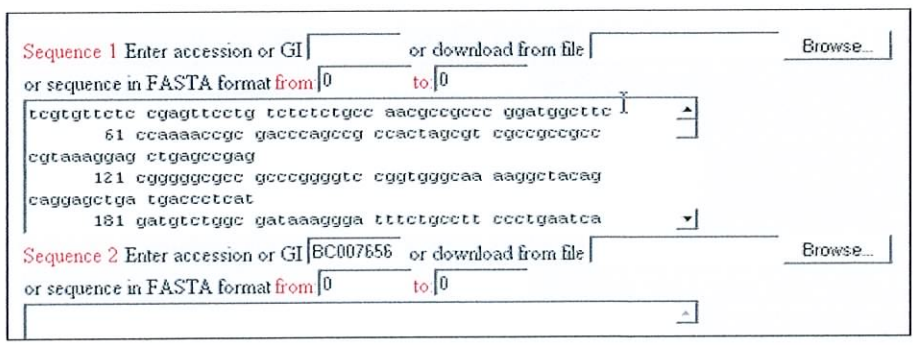

Figure 3. Pairwise BLAST (webshot courtesy of the NCBI).

By Restriction Digestion

To locate the restriction enzymes used to construct a particular clone, see the Clone Details screen (by clicking "View" in the clone query results screen) and look under Library Info. This section contains all available information about how each cDNA was cloned, which may be insufficient to accurately interpret a restriction digest.

- If the clone was constructed using a common cutting restriction enzyme, please consider using an alternate enzyme to ensure that your insert is not being cut as well.
- The construction description may reveal that one or both restriction sites were disrupted upon insertion. In this case, you will need to choose alternate restriction enzymes.
- A helpful restriction mapping tool is located at www.restrictionmapper.org
- Vector maps and sequences for some vectors may be downloaded from the product page on our website.

Making A Stock Culture

Once the clone has been streak isolated and the identity of the strain has been confirmed, we recommend making a stock of the pure culture. Grow the pure culture in LB broth with the appropriate antibiotic. Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at –80°C.

Useful Website

National Center for Biotechnology Information

http://www.ncbi.nlm.nih.gov/

Plate Replication Protocol

Table 3. Materials for plate replication

Item	Vendor	Catalog #
LB-Lennox Broth (low salt)	VWR	EM1.00547.0500
Glycerol	VWR	EM-4760
Ampicillin	VWR	EM-2200
Chloramphenicol	VWR	EM-3130
Kanamycin	VWR	80058-286
96-well microplates	VWR	62407-174
Aluminum seals	VWR	73520-056
Disposable replicators	Genetix	X5054

Procedure

Prepare Target Plates

- Dispense ~160 µl of sterile LB media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates

- Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a Kimwipe dampened with alcohol.

Replicate

- Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
- Gently remove the replicator from the source plate and gently place the replicator into the target plate. Gently move the replicator back and forth in the target plate to transfer cells.
- Discard the replicator.
- Place the lids back on the source plates and target plates.
- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the –80°C freezer.
- Place the inoculated target plates in a 37°C incubator. Incubate the plates for 12–24 hours.

FAQS/Troubleshooting

For answers to questions that are not addressed here, please email technical support at openbiosystems@thermofisher.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

Image Consortium Good Faith Agreement (revised 9/00)

Agreement In Good Faith Concerning Use And Distribution Of Arrayed Cdna Clones

You are being provided with IMAGE Consortium [LLNL] cDNA clones (CLONES) and/or associated products (PRODUCTS) (referred to collectively as "IMAGE MATERIALS"), in order to advance the public interest and to advance the objectives of the institutions that developed the original libraries from which these clones were derived (Originators). The Originators are the beneficiaries of, and may independently enforce, this Agreement.

Use Of Image Materials

By accepting IMAGE MATERIALS you are agreeing in good faith to the following terms. If you are unable to agree to these terms, you must immediately return IMAGE MATERIALS along with all copies and replicas thereof.

(a) You will use the IMAGE MATERIALS in compliance with all applicable laws, governmental regulations and guidelines, including National Institutes of Health guidelines or their equivalent, and any regulations or guidelines pertaining to research with humans, or animals, or with recombinant DNA.

(b) You may use CLONES to produce PROGENY, and to create DERIVATIVE PRODUCTS. You may use IMAGE MATERIALS, PROGENY, and DERIVATIVE PRODUCTS for commercial or non-commercial purposes, except for the purpose of redistribution of CLONES or PROGENY. Accordingly, you may transfer CLONES or PROGENY to additional parties only if 1) this document in its entirety accompanies CLONES or PROGENY, and 2) you transfer CLONES or PROGENY at no cost to such additional parties. "PROGENY" means an unmodified descendant from CLONES or any comparable bacterial stock derived from CLONES (STOCK). "DERIVATIVE PRODUCTS" means any modification or product of CLONES or PRODUCTS that is not a PROGENY or a STOCK.

(c) You will include the unique and specific identifier of each arrayed clone (which was initially assigned by Lawrence Livermore National Laboratory, Livermore, California, and accompanies the IMAGE MATERIALS) in data pertaining to the IMAGE MATERIALS submitted to public databases and in resulting publications. This nomenclature consists of the term "IMAGE Consortium CloneID" followed by a five to seven digit number. You will refer publicly (including but not limited to electronic and print versions of articles and databases) to these arrayed cDNA clones as the "IMAGE Consortium [LLNL] cDNA Clones", and will reference the following publication: "The IMAGE Consortium: An Integrated Molecular Analysis of Genomes and their Expression," Lennon, G.G., Auffray, C., Polymeropoulos, M., and Soares, M.B. [1996] Genomics 33, pgs. 151-152. In INTERNET/World Wide Web publications and databases, you agree to provide electronic referencing (e.g. 'anchors' and/or 'hotlinks') to the IMAGE Consortium home page, currently located at URL http://image.llnl.gov.

(D) you agree that the image materials are experimental in nature and are being provided without warranty, express or implied, including any implied warranty of merchantability or fitness for a particular purpose or freedom from infringement of any patent or other proprietary right of a third party.

(E) you agree to hold harmless and indemnify the regents of the university of california, lawrence livermore national laboratory, the department of energy, the u.S. Government, the originators of the library from which clones were arrayed, the provider of the image materials and persons acting on their behalf, for any claim asserted by a third party related to your possession, use, storage, or disposal of the image materials.

(f) You understand that the ownership of the unarrayed cDNA libraries from which clones were arrayed is retained by the Originators of those libraries. Any new patentable developments or inventions first made by any party using the arrayed clones will remain the property of the inventing party. This Agreement does not constitute the Originators' waiver of any patent rights.

Administration

Any correspondence concerning this Agreement should be addressed to:
Lawrence Livermore National Laboratory
The Regents of the University of California
Industrial Partnerships and Commercialization Program
Attn: IMAGE Consortium
P.O. Box 808, L-795
Livermore, CA 94550
Phone: (925) 422-6416
Fax: (925) 423-8988
http://image.llnl.gov/image/html/GFA.shtml

Incyte cDNA Clones Limited Use License

This limited license permits the person or legal entity to which this product has been provided to use the product and the data generated by the use of this product, only for its internal research purposes. In addition, Buyer acknowledges and agrees that it is not licensed to (a) provide commercial data or databases to or on behalf of any third party that relate to the use of the product; or (b) use the product or data therefrom in a clinical diagnostic setting where data resulting from the testing of an individual's sample is given to such individual or their physician or caregiver. Licensor and its licensors expressly disclaim any warranties of non-infringement. Neither Licensor or its licensors grants any other licenses, express or implied to permit the manufacture, use, sale or importation of this product.

Questions regarding this Limited Label License can be addressed to licensing@openbiosystems.com

Contact Information
Technical Support
Tel: 1.888.412.2225
Fax: 1.256.704.4849
openbiosystems@thermofisher.com

ATTACHEMENTS

Public Health Agency of Canada | Agence de la santé publique du Canada

Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3rd Ed., 2004*

This letter serves to confirm that the Office of Laboratory Security has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

Organization:	University of Western Ontario Robarts Research Institute
Attention:	Dr. Michael Rieder
Address:	100 Perth Drive London, ON N6A 5K8
Laboratory Room Number(s):	2220
Type of work:	■ *in vitro* only ☐ *in vitro* and *in vivo**
Compliance Letter expiry date:	July 28, 2011.

To renew your compliance letter please complete a CL2 checklist and fax it to our office at (613) 941-0596. The checklist can be obtained from the following website: www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz
Chief, Importation and Regulatory Affairs

SEPTEMBER 23, 2009
Date

*The Office of Laboratory Security must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses.

Canada

Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

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

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

Laboratory Compliance to *Containment Standards for Veterinary Facilities*

The Office of Biohazard Containment and Safety (OBCS) has received and reviewed the Inspection Checklist for the Animal Pathogen Containment Level 2 Facility below. This letter serves to confirm the OBCS has found the information provided to be **acceptable for work *in vitro***.

Organization:	The University of Western Ontario Robarts Research Institute
Address:	100 Perth Drive London, Ontario N6A 5K8
Attention:	Anda Marcu, Dr. Michael J. Rieder and Ron Noseworthy
Phone Number:	519-931-5777
Laboratories:	Rooms 2226 and 2220
CFIA Compliance Number:	C-2010-0033-4
Compliance Letter expiry date:	January 14, 2012

For your reference, the *Containment Standards for Veterinary Facilities*, from which the inspection checklist was adapted, are available on the internet at the following address: http://www.inspection.gc.ca/english/sci/bio/bioe.shtml. Please visit our website for more information and updates on our program.

Note: Canadian distributors of biological products (animal pathogens) regulated under the *Health of Animals Act* will require their clients to submit a copy of this letter.

Please do not hesitate to contact the Office of Biohazard Containment and Safety of the CFIA if you have any questions regarding this letter.

Sincerely,

Cinthia Labrie

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

20 JAN. 2010
Date

Canada

Bacteria

ATCC® Number: **17857™** Order this Item Price: **$255.00**

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

Organism:	*Clostridium difficile* (Hall and O'Toole) Prevot
Designations:	870
Depositor:	LS McClung
Biosafety Level:	2
Shipped:	freeze-dried
Growth Conditions:	ATCC medium1080: Schaedler broth Alternate medium 260: Trypticase soy agar with defibrinated sheep blood Alternate medium 38: Beef liver medium for anaerobes **Temperature:** 37.0°C **Atmosphere:** Anaerobic
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.
Comments:	Presence of *tcdA* and *tcdB* genes confirmed by PCR.
References:	8131: Delmee M, et al. Serogrouping of Clostridium difficile strains by slide agglutination. J. Clin. Microbiol. 21: 323-327, 1985. PubMed: 3980688 16173174: Lemee L, et al. Multiplex PCR targeting tpi (triose phosphate isomerase), tcdA (Toxin A), and tcdB (Toxin B) genes for toxigenic culture of Clostridium difficile. J. Clin. Microbiol. 42(12): 5710-5714, 2004. PubMed: 15583303

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

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Clostridium difficile - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Clostridium difficile***

SYNONYM OR CROSS REFERENCE: N/A

CHARACTERISTICS: Gram positive rod, anaerobic, motile, subterminal spores, produces a cytotoxin and enterotoxin

SECTION II - HEALTH HAZARD

PATHOGENICITY: Opportunistic pathogen, broad-spectrum antibiotic therapy eliminates competing gut flora, allowing the overgrowth of *C. difficile*; important cause of antibiotic-associated diarrhea and pseudomembranous colitis; diarrhea in cancer patients receiving chemotherapy; symptoms range from mild diarrhea to severe colitis (possibly fatal)

EPIDEMIOLOGY: Worldwide; 2-3% of adults are asymptomatic carriers ; 50% of healthy neonates (<1 year old) are carriers; nosocomial transmission increasingly important

HOST RANGE: Humans and other animals

INFECTIOUS DOSE: Not known

MODE OF TRANSMISSION: Fecal-oral contact; evidence for transmission via fomites and hands exists

INCUBATION PERIOD: Not known

COMMUNICABILITY: May be transmitted from person to person

SECTION III - DISSEMINATION

RESERVOIR: Soil, water, hay, sand; intestinal tract of humans and other animals

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Susceptible to metronidazole and vancomycin

DRUG RESISTANCE: Metronidazole and vancomycin-resistant strains have been reported

SUSCEPTIBILITY TO DISINFECTANTS: Spores are fairly resistant; moderate susceptibility to 1% sodium hypochlorite; susceptible to high level disinfectants (>2% glutaraldehyde) with prolonged contact time

PHYSICAL INACTIVATION: Spores are fairly resistant to heat (spores destroyed by moist heat - 121°C for at least 15 min)

SURVIVAL OUTSIDE HOST: Spores can survive for long periods outside of host

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; recovery of *C. difficile* organisms and/or toxin from stool samples

FIRST AID/TREATMENT: Antibiotic therapy should be stopped; oral therapy with metronidazole or vancomycin

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 1 reported case of a laboratory-acquired infection from *C. difficile*

SOURCES/SPECIMENS: Clinical specimens - feces

PRIMARY HAZARDS: Injuries from contaminated sharp instruments

SPECIAL HAZARDS: Not known

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and cultures

PROTECTIVE CLOTHING: Laboratory coat; gloves when direct contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wear protective clothing; gently cover spill with paper towels and apply a suitable disinfectant (high level or 1% sodium hypochlorite), starting at perimeter and working towards the centre; allow sufficient contact time before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection, incineration

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: January 2000

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2010-06-02

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

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

Canada

Info on cell line(s)

Cell Biology

ATCC® Number:	**TIB-152™** Order this Item	Price:	**$272.00**

Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast 
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]
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)
Receptors:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.
Gender:	male

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Comments:	This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609] The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530] Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609] The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. 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:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml. **Interval:** Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-1990 derivative:ATCC CRL-2063 derivative:ATCC TIB-153

References:

1609: Weiss A, et al. The role of T3 surface molecules in the activation of human T cells: a two-stimulus requirement for IL-2 production reflects events occurring at a pre-translational level. J. Immunol. 133: 123-128, 1984. PubMed: 6327821
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32704: Chan YJ, et al. Synergistic interactions between overlapping binding sites for the serum response factor and ELK-1 proteins mediate both basal enhancement and phorbol ester responsiveness of primate cytomegalovirus. J. Virol. 70: 8590-8605, 1996. PubMed: 8970984
32755: Kung SH, Medveczky PG. Identification of a herpesvirus saimiri cis-acting DNA fragment that permits stable replication of episomes in transformed T cells. J. Virol. 70: 1738-1744, 1996. PubMed: 8627695
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32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: 8855306
32904: Linette GP, et al. Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation.

ScienCell™
Research Laboratories

Human Cardiac Myocytes (HCM)

Catalog Number: 6200

Cell Specification

The cardiac myocyte is the most physically energetic cell in the body. Its contraction is myogenic, i.e. it is independent of nervous stimulation. All cardiac myocytes are capable of spontaneous rhythmic depolarization and repolarization of their membrane. Cardiac myocytes occupy as much as 75% of cardiac mass but constitute only about one third of the total cell number in the heart. They are highly specialized high-oxygen-content cells and house a large number of mitochondria [1]. Differentiated cardiac myocytes have little capacity to proliferate and show the hypertrophic growth in response to alpha1-adrenergic stimuli via the Ras/MEK pathway [2]. Cardiac myocyte hypertrophy and apoptosis have been implicated in the loss of contractile function during heart failure. Cardiac myocytes have a complex network of signals that regulates their essential role in the rhythmic pumping of the heart [3]. This network is an appealing model system in which to study the basic principles of cellular signaling mechanisms leading to cardiac myocyte death.

HCM from ScienCell Research Laboratories are isolated from the human heart (ventricle). HCM are cryopreserved immediately after purification and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HCM are characterized by immunofluorescent method with antibodies to myosin. HCM are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HCM are guaranteed to further culture at the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Cardiac Myocyte Medium (CMM, Cat. No. 6201) for the culturing of HCM *in vitro*.

Product Use

HCM are for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Directly and immediately transfer cells from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture needed for experiments.

Shipping

Dry ice.

Reference

[1] Bodyak, N., Kang, P. M., Hiromura, M., Sulijoadikusumo, I., Horikoshi, N., Khrapko, K. and Usheva, A. (2002) Gene expression profiling of the aging mouse cardiac myocytes. *Nucleic Acids Research* 30(17):3788-3794.

[2] Tamamori-Adachi, M., Ito, H., Nobori, K., Hayashida, K., Kawauchi, J., Adachi, S., Ikeda, M. A. and Kitajima, S. (2002) Expression of cyclin D1 and CDK4 causes hypertrophic growth of cardiomyocytes in culture: a possible implication for cardiac hypertrophy. *Biochem Biophys Res Commun* 296(2):274-80.

[3] Sambrano, G.R., Fraser, I., Han, H., Ni, Y., O'Connell, T., Yan, Z. and Stull, J. T. (2002) Navigating the signaling network in mouse cardiac myocytes. *Nature* 420(6916):712-4.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the ordering:

1. Prepare a poly-L-lysine coated flask (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently re-suspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm^2 is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that HMC are plated in poly-L-lysine coated flask that promotes cell attachment and growth.
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

Subculture:

HCM are not recommended to be subcultured since this cell type is going to terminally differentiate soon in culture. The following is only for reference in case needed:

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks (2 $\mu g/cm^2$).
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS (Ca^{++} and Mg^{++} free, cat. no. 0303) to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, cat. no. 0500); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 or 2 minutes more (no solution in the flask at this moment); at the end of trypsinisation, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (*Beckman Coulter* Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling human derived products is potentially hazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Poh, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).

Cell Biology

ATCC® Number:	**CRL-2190™** Order this Item	Price:	**$272.00**

Designations:	HK-2
Depositors:	RA Zager
Biosafety Level:	2 [Cells Contain Papilloma viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney, cortex **Tissue:** proximal tubule **Cell Type:** human papillomavirus 16 (HPV-16) transformed
Cellular Products:	alkaline phosphatase; gamma glutamyltranspeptidase; leucine aminopeptidase; acid phosphatase; cytokeratin; alpha 3, beta 1 integrin; fibronectin
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.
Receptors:	epidermal growth factor (EGF), expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 13 D13S317: 9 D16S539: 11,12 D5S818: 12 D7S820: 10,11 THO1: 9 TPOX: 8,9 vWA: 17,18
Age:	adult
Gender:	male

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HK-2 (human kidney 2) is a proximal tubular cell (PTC) line derived from normal kidney.
The cells were immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes.
The recombinant retrovirus vector pLXSN 16 E6/E7 containing the HPV-16 E6/E7 genes was used to transfect the ectotropic packaging cell line Psi-2.
Virus produced by the Psi-2 cells was used to infect the amphotropic packaging cell line PA317 (see ATCCCRL-9078).
Virus produced by the PA317 cells was used to transduce primary PTCs.
Although pLXSN 16 E6/E7 also confers resistance to neomycin, selection in G418 was not used to isolate transduced clones.
The cell line appears to be derived from a single cell based on Southern and FISH analysis.
The E6/E7 genes are present in the HK-2 genome as determined by PCR.

Comments:

The cells retain a phenotype indicative of well differentiated PTCs.
They are positive for alkaline phosphatase, gamma glutamyltranspeptidase, leucine aminopeptidase, acid phosphatase, cytokeratin, alpha 3,beta 1 integrin, and fibronectin.
The cells are negative for factor VIII related antigen, 6.19 antigen and CALLA endopeptidase.
HK-2 cells retain functional characteristics of proximal tubular epithelium such as Na+ dependent / phlorizin sensitive sugar transport and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic hormone.
The cells are capable of gluconeogenesis as evidenced by their ability to make and store glycogen.
HK-2 cells are anchorage dependent.
The cells will not grow in methylcellulose, soft agar or suspension.
HK-2 cells can reproduce experimental results obtained with freshly isolated PTCs.

Propagation:

ATCC complete growth medium: The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:

- 0.05 mg/ml BPE - provided with the K-SFM kit
- 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: Cell growth is dependent on epidermal growth factor. The cells should not be allowed to become confluent. Subculture at 80% of confluence.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.05% (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. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:4 is recommended
Medium Renewal: Every 2 to 3 days
Freeze medium: Complete growth medium supplemented with 7.5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

References:

22466: Ryan MJ, et al. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. Kidney Int. 45: 48-57, 1994. PubMed: 8127021

Cell Biology

ATCC® Number:	**HB-8065™**	Order this Item	Price:	**$272.00**

Designations:	Hep G2
Depositors:	Wistar Institute
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; [3525] complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein) [3525]
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)
Receptors:	insulin; insulin-like growth factor II (IGF II) [22446]
Tumorigenic:	No

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DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 F13A01: 5,7 F13B: 6,10 FESFPS: 11 LPL: 10,11 THO1: 9 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	modal number = 55 (range = 50 to 60); has a rearranged chromosome 1 [3525]
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. [23557] The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to gramoxone (oxidative stress). [26594] There is no evidence of a Hepatitis B virus genome in this cell line. [1205] [22909]
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 **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%

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.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: Twice per week

Preservation:

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 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CRL-10741
derivative:ATCC CRL-11997
purified DNA:ATCC HB-8065D

References:

1205: Knowles BB, et al. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. Science 209: 497-499, 1980. PubMed: 6248960
3525: Knowles BB, Aden DP. Human hepatoma derived cell line, process for preparation thereof, and uses therefor. US Patent 4,393,133 dated Jul 12 1983
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23557: Busch SJ, et al. Differential regulation of hepatic triglyceride lipase and 3-hydroxy-3- methylglutaryl-CoA reductase gene expression in a human hepatoma cell line, HepG2. J. Biol. Chem. 265: 22474-22479, 1990. PubMed: 2176219
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32564: Roesler WJ, et al. The alpha-isoform of the CCAAT/enhancer-binding protein is required for mediating cAMP responsiveness of the phosphoenolpyruvate

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