

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

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:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
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 HCM.

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 assess 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? YES 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? YES NO
 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
<i>C. difficile</i>	<input type="checkbox"/> X Yes <input type="checkbox"/> No	<input type="checkbox"/> X Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> X No	- *	ATCC	<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>E. coli</i> <i>DT103 TnA</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	* *		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

*Host strain
DT103 TnA*

*Please attach a Material Safety Data Sheet or equivalent from the supplier.
** Not culturing any E. coli at the moment. We have no S. sul freeze dried.*
2.0 Cell Culture *** Not culturing any E. coli at the moment.*

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	<input checked="" type="checkbox"/> X Yes <input type="checkbox"/> No	ATCC, Science Cell <i>FBL - Human Voluntary</i>	Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> X No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> X No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> X No		

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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Jurkat E 6.1; Human Cardiomyocytes; HK-2, HepG2	ATCC, ScienCell
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		

*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 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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid	N/A	<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	N/A	<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 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? 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 transfection
<i>E. coli</i> <i>E. coli</i> DH10B Ton A	pJ3omega-MDR3 SLC28A3_Human	ATCC	ABC(B ₄) gene SLC28A3	transfected Human Cardiomyocytes DH10B Ton A

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

** Please attach a plasmid map.

Host strain
DH10B Ton A
Add a
transgene
(ABC(B₄))

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 HIV-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 NO
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
 Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? 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 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 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 LD₅₀ (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 XNO 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: _____
 "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 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 "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? 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? YES NO

11.4 Has the import permit been sent to OHS? 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: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

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

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Plasmid(s)

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

DNA: cDNA

Insert lengths(kb): 4.0

Tissue: liver

Insert Information:

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](#)]

Size (kb): 3.5000000000000000

Vector: pJ3omega (plasmid)

Promoters: Promoter SV40 early

Construction: pBR322, SV40

Marker(s):ampR

Construct size (kb): 3.5

Vector:

Features: marker(s): ampR

other: SV40 t IVS

promoter: SV40 early

replicon: SV40

replicon: pMB1

MCS: HindIII...BglII

terminator: SV40

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Restriction digests of the clone give the following sizes (kb):
HindIII/XbaI--4.0, 3.5; BamHI--4.5, 3.0; Aval--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](#)]

Comments:

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

27333: van der Bliet AM, et al. Sequence of mdr3 cDNA
encoding a human P-glycoprotein. Gene 71: 401-411, 1988.
PubMed: [2906314](#)

References:

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

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 be 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).

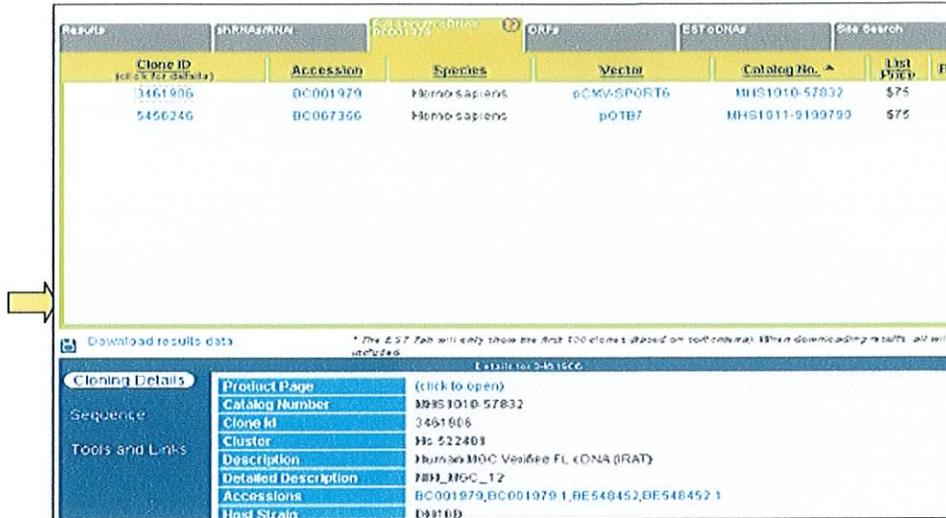


Figure 2. Thermo Scientific Open Biosystems Gene Query Details

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).

Sequence 1 Enter accession or GI or download from file Browse...

or sequence in FASTA format from to

```
tcgtgtcttc cgagttcctg tctctctgcc aagccgccc ggatgcttc
61 ccaaaaccgc gaccacgccc ccaactagcgt cgcgcgcgcc
cgtaaaggag ctgagccgag
121 cggggggccc gccgggggtc cggtgggcaa aaggctacag
caggagctga tgaccctcat
181 gatgtctggc gataaaggga tttctgcctt cctgaaatca
```

Sequence 2 Enter accession or GI or download from file Browse...

or sequence in FASTA format from to

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 μ L of culture into a polypropylene tube and add 80 μ L 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 μ L 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.

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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.

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(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>

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Contact Information

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

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ATTACHEMENTS

Public Health
Agency of CanadaAgence 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

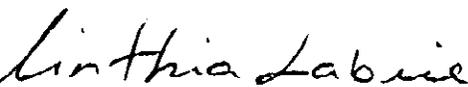
Compliance Letter expiry date: January 14, 2012

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

20 JAN. 2010

Date

Canada

MSDS'

Bacteria

ATCC® Number:

17857™

[Order this Item](#)

Price:

\$255.00

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Organism:

Clostridium difficile (Hall and O'Toole) Prevot

Designations:

870

Depositor:

LS McClung

Biosafety Level:

2

Shipped:

freeze-dried

[ATCC medium 1080](#): Schaedler broth[Alternate medium 260](#): Trypticase soy agar with defibrinated sheep blood

Growth Conditions:

[Alternate medium 38](#): Beef liver medium for anaerobes**Temperature:** 37.0°C**Atmosphere:** Anaerobic

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.

Permits/Forms:

Comments:

Presence of *tcdA* and *tcdB* genes confirmed by PCR.

8131: Delmee M, et al. Serogrouping of *Clostridium difficile* strains by slide agglutination. J. Clin. Microbiol. 21: 323-327, 1985. PubMed: [3980688](#)

References:

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](#)

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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

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.

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Date Modified: 2010-06-02



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Escherichia coli, enterohemorrhagic - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Escherichia coli*, enterohemorrhagic

SYNONYM OR CROSS REFERENCE: Enterohemorrhagic *Escherichia coli* (EHEC), Verotoxin producing *Escherichia coli* (VTEC), Shiga toxin producing *Escherichia coli* (STEC)

CHARACTERISTICS: Gram negative rod; motile, aerobic; produce Vero / Shiga toxins (VT/STx), 2 types, VT1/Stx1 and VT2/Stx2; serotyping to determine somatic and flagellar antigens

SECTION II - HEALTH HAZARD

PATHOGENICITY: Hemorrhagic colitis, intestinal disease accompanied by cramps and abdominal pain; initially watery, followed by bloody diarrhea; low grade fever; last about 8 days; 5-10% of hemorrhagic colitis victims may develop hemolytic uremic syndrome (HUS); affects all ages, higher death rates occur in elderly and young; can cause thrombocytopenic purpura (TTP) in elderly

EPIDEMIOLOGY: Sporadic and in outbreaks of bloody diarrhea; associated with 15-30% of patients where no other pathogen has been identified; main EHEC serotype in North America from infections is *E. coli* O157:H7

HOST RANGE: Humans; animals (O157:H7 - piglets, calves and cattle)

INFECTIOUS DOSE: Appears to have low infectious dose, may be similar to that of *Shigella* spp., 10 organisms by ingestion

MODE OF TRANSMISSION: Ingestion of contaminated food (undercooked hamburger meat, unpasteurized milk); fecal-oral transmission; person-to-person transmission (extremely high)

INCUBATION PERIOD: 2-8 days (median of 3-4 days)

COMMUNICABILITY: Communicable for duration of fecal excretion (7-9 days); 3 weeks in one third of children

SECTION III - DISSEMINATION

RESERVOIR: Infected persons, animals (sheep, goats, pigs, poultry, calves, cattle)

ZOONOSIS: Yes - direct or indirect contact with infected animal and waste

VECTORS: birds may be a vector

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to a wide spectrum of antibiotics

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 70% ethanol, phenolics, glutaraldehyde, iodines, formaldehyde

PHYSICAL INACTIVATION: Heat sensitive, inactivated by moist heat (121° C for at least 15 min) and dry heat (160-170° C for at least 1 hour)

SURVIVAL OUTSIDE HOST: Butter - up to 50 min; cream - 10 days; hamburger meat - survives well; does not survive long in slurry systems (innoculum of 10⁸ cfu/mL became undetectable after 9 days); survives well in contaminated feces and soil, only small reduction in organism number over 2 months

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm bacteriologically, DNA probe to detect Verotoxins VT1 and VT2

FIRST AID/TREATMENT: Electrolyte fluid therapy; antibiotics may be administered in very severe cases

IMMUNIZATION: None

PROPHYLAXIS: Not usually administered

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 4 reported cases of laboratory infections with *E. coli* since 1981

SOURCES/SPECIMENS: Contaminated food (raw milk, hamburger, apple juice and water); feces

PRIMARY HAZARDS: Ingestion

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

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

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

OTHER PRECAUTIONS: Good personal hygiene and frequent handwashing essential

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: January, 2001

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.

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Date Modified: 2001-09-27



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

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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 considered to be level 1 animal pathogens:

• 5K	• CIE85	• J52	• MC4100 (MuLac)	• U5/41
• 58	• DH1	• J53	• MG1655	• W208
• 58-161	• DH10 GOLD	• JC3272	• MM294	• W945
• 679	• DH10B	• JC7661	• MS101	• W1485
• 1532	• DH5	• JC9387	• NC-7	• W3104
• AB284	• DH5-alpha	• JF1504	• Nissle 1917	• W3110
• AB311	• DP50	• JF1508	• One Shot STBL3	• WA704
• AB1157	• DY145	• JF1509	• OP50	• WP2
• AB1206	• DY380	• JJ055	• P678	• X1854
• AG1	• E11	• JM83	• PA309	• X2160T
• B	• EJ183	• JM101	• PK-5	• X2541
• BB4	• EL250	• JM109	• PMC103	• X2547T
• BD792	• EMG2	• K12	• PR13	• XL1-BLUE
• BL21	• EPI 300	• KC8	• Rri	• XL1-BLUE-MRF
• BL21 (DE3)	• EZ10	• KA802	• RV308	• XL0LR
• BM25.8	• FDA Seattle 1946	• KAM32	• S17-1λ -PIR	• Y10
• C	• Fusion-Blue	• KAM33	• SCS1	• Y1090 (1090)
• C-1a	• H1443	• KAM43	• SMR10	• YN2980
• C-3000	• HF4714	• LE450	• SOLR	• W3110
• C25	• HB101	• LE451	• SuperchargeEZ10	• WG1
• C41 (DE3)	• HS(PFAMP)R	• LE452	• SURE	• WG439
• C43 (DE3)	• Hfr3000	• MB408	• TOP10	• WG443
• C600	• Hfr3000 X74	• MBX1928	• TG1	• WG445
• Cavalli Hfr	• HMS174	• MC1061		

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,

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

Info on cell line(s)

ils/t...

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)
lymphoblast

Morphology:  PHOTO

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

Amelogenin: X,Y

CSF1PO: 11,12

D13S317: 8,12

D16S539: 11

DNA Profile (STR): 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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- 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](#)
- 23430: Gillis S, Watson J. Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2-producing human leukemia T cell line. *J. Exp. Med.* 152: 1709-1719, 1980. PubMed: [6778951](#)
- 32253: Berninghausen O, Leippe M. Necrosis versus apoptosis as the mechanism of target cell death induced by *Entamoeba histolytica*. *Infect. Immun.* 65: 3615-3621, 1997. PubMed: [9284127](#)
- 32368: Churchill MJ, et al. The rev-responsive element negatively regulates human immunodeficiency virus type 1 env mRNA expression in primate cells. *J. Virol.* 70: 5786-5790, 1996. PubMed: [8709194](#)
- 32396: Kolanus W, et al. alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule. *Cell* 86: 233-242, 1996. PubMed: [8706128](#)
- 32446: Gan W, Rhoads RE. Internal initiation of translation directed by the 5'-untranslated region of the mRNA for eIF4G, a factor involved in the picornavirus-induced switch from cap-dependent to internal initiation. *J. Biol. Chem.* 271: 623-626, 1996. PubMed: [8557663](#)
- 32561: Tiffany HL, et al. Enhanced expression of the eosinophil-derived neurotoxin ribonuclease (RNS2) gene requires interaction between the promoter and intron. *J. Biol. Chem.* 271: 12387-12393, 1996. PubMed: [8647842](#)
- 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](#)
- 32796: Bloom TJ, Beavo JA. Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. *Proc. Natl. Acad. Sci. USA* 93: 14188-14192, 1996. PubMed: [8943082](#)
- 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.

References:



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 $>5 \times 10^6$ 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] Boydak, N., Kang, P. M., Hironuma, M., Suljodikusumo, 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-Abechi, M., Ito, H., Nobori, K., Hayashida, K., Kawachi, 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] Sambroano, 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 $\mu\text{g}/\text{cm}^2$, 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/cm² 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 HCM 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 ($2 \mu\text{g}/\text{cm}^2$).
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.

1. Centrifuge tube (harvested cell suspension) at 1000 rpm (Beckman) 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 biohazardous. Although each cell strain tests 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 Pelt, 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
alkaline phosphatase; gamma glutamyltranspeptidase;
Cellular Products: 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
Amelogenin: X,Y
CSFIPO: 13
D13S317: 9
D16S539: 11,12

DNA Profile (STR): 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 ATCC [CRL-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.

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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:

Propagation:

- 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.

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.

Subculturing:

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

Medium Renewal: Every 2 to 3 days

Preservation:

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)
epithelial

Morphology: 

Source: **Organ:** liver
Disease: hepatocellular carcinoma
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]

Cellular Products: 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
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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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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 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](#)

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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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