Modification Form for Permit B10 0HO 0 0 10

Permit Holder: Daniel Hardy

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

Lin Zhao

Jessica Osumek

Gurjeev Sohi

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	Rodent (primary) liver, Rodent (established) H4TG	→ human (established), FL 62891 human fetal liver cell line, ↳ clone 9 rat neonatal hepatocytes
Approved Use of Human Source Material		
Approved GMO		
Approved use of Animals		

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

Classification: ✗ 2 → PSB 2018 inspected Feb 6/09 gf.

Date of last Biohazardous Agents Registry Form: May 26, 2008

Signature of Permit Holder Dan Hardy

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

Approved Toxin(s)

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

Classification: + 2

Date of last Biohazardous Agents Registry Form: May 26, 2008

Signature of Permit Holder Da Hardy

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

My laboratory examines the mechanisms underlying the early origins of adult disease. Specifically, I am interested on how nuclear receptors and epigenetic mechanisms play a role the development of intrauterine growth restriction (IUGR), and furthermore, cardiovascular disease into adulthood. I have a particular emphasis on how hypoxia and nutrition (e.g. low protein diet) in pregnancy leads to elevated cholesterol into adulthood. To examine this further, I have an in vivo low protein rat pregnant rat model as well as in vitro liver hepatocytes (of human and rat origin) to examine the underlying mechanisms further.

I hope this helps. Thanks again for your assistance last Friday!

Cheers,

Dan

Daniel B. Hardy, PhD
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Departments of Obstetrics and Gynaecology
& Physiology and Pharmacology
Schulich School of Medicine and Dentistry
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Product Description

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Cell Biology

ATCC® Number:	**CRL-11005™** Order this Item	**Price:**	**$353.00**
Designations:	FL 62891	**Depositors:**	ImClone Systems Inc.
Biosafety Level:	2 [Cells contain SV-40 viral DNA sequences]	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	fibroblast
			PHOTO

Source: **Organ:** liver
Cell Type: immortalized with SV40 large T antigen

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.

This material is cited in a U.S. and/or other Patent or Patent Application, and may not be used to infringe on the patent claims. ATCC is required to inform the Patent Depositor of the party to which the material was furnished.

Related Cell Culture Products

Age: fetus

Comments: The cell line was derived from human fetal liver tissue, and immortalized by transformation with an ecotropic retrovirus containing a temperature sensitive SV40 T-antigen (tsA58) and a G418 resistance gene. After several days at 37C, the temperature of the medium was lowered to 32C. Cells were selected with G418. The selected cells were expanded and maintained. The cells produce flk-1 and some flk-2 ligands.

Propagation: **ATCC complete growth medium:** Iscove's modified Dulbecco's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate supplemented with 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, and 15% fetal bovine serum
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 32.0°C

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 which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 32°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 32°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Doubling Time: 48 hours

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2005
recommended serum:ATCC 30-2020

References:
70742: Lemischka IR. Nucleic acids encoding fragments of hematopoietic stem cell receptor flk-2. US Patent 5,270,458 dated Dec 14 1993
70744: Lemischka IR. Tyrosine kinase receptor flk-2 and fragments thereof. US Patent 5,367,057 dated Nov 22 1994
70745: Lemischka IR. Tyrosine kinase receptor human flk-2-specific antibodies. US Patent 5,548,065 dated Aug 20 1996
70746: Lemischka IR. Nucleic acids encoding soluble human FLK-2 extracellular domain. US Patent 5,621,090 dated Apr 15 1997
70747: Lemischka IR. Antibodies against tyrosine kinase receptor flk-1. US Patent 5,747,651 dated May 5 1998
70748: Lemischka IR. Method for isolating stem cells expressing flk-1 receptors. US Patent 5,912,133 dated Jun 15 1999

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Product Description

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Cell Biology

ATCC® Number:	**CRL-1439™** Order this Item	**Price:**	**$323.00**
Designations:	clone 9	**Depositors:**	ME Kaighn
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	Rattus norvegicus (rat)	**Morphology:**	epithelial

Source: **Organ:** liver
Strain: Sprague-Dawley
Disease: normal

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

Related Cell Culture Products

Isolation: **Isolation date:** 1968

Age: 4 weeks

Gender: male

Comments: Clone 9 (K-9) is an epithelial cell line isolated in 1968 from normal liver taken from a young male rat.
The line has been used for studies of in vitro carcinogenesis and is useful clonal assays for screening sera and other nutritional supplements.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing: **Protocol:**

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 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004

recommended serum:ATCC 30-2020

References:

21872: . Gene expression and carcinogenesis in cultured liver. New York: Academic Press; 1975.
22425: Weinstein IB, et al. Growth and structural properties of epithelial cell cultures established from normal rat liver and chemically induced hepatomas. Cancer Res. 35: 253-263, 1975. PubMed: 162864

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ATCC products are intended for laboratory research purposes only, unless noted otherwise. They are not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this site, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

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BIO - UWO - 0210

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans. This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

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

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

PRINCIPAL INVESTIGATOR Daniel Hardy
SIGNATURE Dan Hardy
DEPARTMENT Obl Gyn
ADDRESS DSB 5006, Department of Physiology Pharmacology
PHONE NUMBER x 84238
EMAIL Daniel.Hardy@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB DSB Room(s) 5006 (Lab) Tissue Culture

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): Role of Nuclear Receptors in Fetal Programming

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

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

Mr. Gurgeev Sohi
Ms. Jessica Osumek.
Dr. Lin Zhao

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent (Frozen) (liver)	☒ Yes O No	Dr. Edith Arany	~~N/A~~
Non-human primate	O Yes O No		N/A
Other (specify)	O Yes O No		N/A

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		N/A
Rodent	☒ Yes O No	H4TG	ATTC
Non-human primate	O Yes O No		N/A
Other (specify)	O Yes O No		N/A

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

2.4 For above named cell types(s) indicate HC or CFIA containment level required ☒ 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ☒ NO
If no, please proceed to Section 4.0

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

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

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

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

5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? _____

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

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

6.0 Animal Experiments

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

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

9.0 Import Requirements

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

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

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

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

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

10.0 Training Requirements for Personnel Named on Form

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

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE Da Hanly

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ○ 2 ○ 3

11.2 Has the facility been certified by OHS for this level of containment?
☒ YES, permit # if on-campus ~~BIO-UWO-0157~~ please see BIO-UWO-0157 (shared w Dr. Tim Regnault)
○ NO
○ NOT REQUIRED

12.0 Procedures to be Followed

12.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Da Hanly Date: MAY 13, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Ickert
Date: 26 May 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: Q Hanley
Date: May 26/08

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

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

Special Conditions of Approval:

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