

**THE UNIVERSITY OF WESTERN ONTARIO
 BIOHAZARDOUS AGENTS REGISTRY FORM**
 Approved Biohazards Subcommittee: September 25, 2009
 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 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 biohazards 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 Biohazard 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 David Hess
 SIGNATURE David Hess
 DEPARTMENT Robarts Research Institute/physiology & pharmacology
 ADDRESS 100 Perth St, London, ON, N6A 5K8
 PHONE NUMBER 519-663-5777 x24152
 EMERGENCY PHONE NUMBER(S) 519-645-2703
 EMAIL dhess@robarts.ca

Location of experimental work to be carried out: Building(s) Robarts Room(s) 4250, 4244A
Robarts Experimental

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for Robarts 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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, Canadian Heart and Stroke Foundation
 GRANT TITLE(S): MOP# 86702 "Formation of a regenerative niche for the recovery of beta cell function", MOP# 86749 "Myokines and cellular constituents and paracrine factors that mediate functional vascular regeneration"

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:
Heather Broughton - Technician
Debbie Robson - Technician
Gillian Bell - Graduate Student
David Putman - Graduate Student
Ridge Thompson - Undergrad Student

1.1 Does your work involve the use of biological agents? YES NO
 (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. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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	PHAC or CFIA Containment Level
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO

If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input checked="" type="radio"/> Yes <input type="radio"/> No	Human bone Marrow Human umbilical cord blood	Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Mouse bone Marrow	2006-126-12 2006-122-12
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

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="radio"/> Yes <input type="radio"/> No	Human Fibroblasts HS 792(C), M	- ATCC
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No	Human Skin Fibroblast HS 707(B), Ep Human Breast Cancer MDA-MB-231	- ATCC - ATCC
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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 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	UCH - St. Joe's Birthing Center BHI - University Hosp.	<input checked="" type="radio"/> Yes <input type="radio"/> No <input checked="" type="radio"/> Unknown	Not Applicable	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input checked="" type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)	Human Bone Marrow University Hospital	<input checked="" type="radio"/> Yes <input checked="" type="radio"/> No <input checked="" type="radio"/> Unknown	Not Applicable	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		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
None - purified DNA only	PGIPZ	Open Biosystems	-shRNA Nodal -shRNA Control	- over inhibition of Nodal expression = tumor cell growth and angiogen - control - no change

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted 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 NOD/SCID, NOD/SCID IL-2R γ null, NOD/SCID/MP5v11

6.3 AUS protocol # 2006-136-12 and 2006- -

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:

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? 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 NO, please forward the permit to the Biosafety Officer when available.

10.9 Please 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 _____
If no, please proceed to Section 12.0 NO

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 # _____ 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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE David Hess

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

13.0 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

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

- YES, permit # if on-campus BIO-RP1-0029
 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 David Head Date: November 12, 2009

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

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

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

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

Formation of a regenerative niche for the endogenous recovery of beta-cell function.

Background. Due to an extreme shortage donor islet tissue available for transplantation via the Edmonton protocol, alternate strategies to support the expansion of insulin-secreting beta-cells are continually sought. However, in the context of regenerative therapies for diabetes the contributions of transplanted stem cells are not limited to direct replacement of damaged cells. We have previously established that transplanted bone marrow cells recruit to damaged islets, induce proliferation in recipient beta-cells, and enhance insulin secretion and glycemic control via the regeneration of endogenous beta-cell function. *However, the essential cellular players and paracrine growth factors that form a regenerative niche for endogenous beta-cell recovery remains largely unidentified.*

Bone marrow (BM) and umbilical cord blood (UCB) contain several primitive cell types that demonstrate regenerative potential after transplantation. However, reliable purification of these rare regenerative progenitors has proven inefficient. We have established unique stem cell isolation procedures using high aldehyde dehydrogenase (ALDH) activity in combination with primitive cell surface markers to select highly-purified hematopoietic, endothelial, and hematopoietic progenitor cells from human BM. On the basis of ALDH-activity we have identified highly-purified human cells that reduce hyperglycemia after transplantation into novel immune-deficient murine recipients. Investigation of the following specific aims will identify human cell lineages that form a “regenerative niche” in the murine pancreas, and will identify conserved paracrine-signaling molecules that mediate the endogenous recovery of beta-cell function.

Specific Aims. (1) To establish human hematopoietic, endothelial, and mesenchymal progenitor cell expansion *in vitro*. ALDH-purified cells from human BM and UCB will establish mesenchymal, endothelial, and hematopoietic expansion cultures and progenitor differentiation will be characterized.

(2) To identify paracrine factors secreted by human ALDH^{hi} mesenchymal, endothelial, or hematopoietic progenitors that augment beta-cell proliferation and insulin production *in vitro*. Putative regenerative progenitor subtypes will be compared for the production of developmental, endocrine, and inflammatory mediators. Co-culture of progenitors with isolated murine islets will document paracrine induction of beta-cell proliferation or insulin production *in vitro*.

(3) To determine which human progenitor lineage(s) are recruited to the pancreas and to identify paracrine factors that mediate the recovery of beta-cell function *in vivo*. We will transplant ALDH-purified progenitor cells, or lineage-restricted expanded progeny (*Aim 1*) to assess the ability of distinct human progenitor cells to induce beta-cell regeneration. Donor cell recruitment to peri-islet regions, endogenous beta-cell proliferation and insulin production, and recovery from hyperglycemia will be assessed in streptozotocin-treated NOD/SCID IL-2R γ null or NOD/SCID/MPSVII mice, novel models for the sensitive detection of transplanted human cells *in situ*. Engrafted cells will be reacquired by FACS and compared for the production of developmental, endocrine, and inflammatory mediators that impact the regeneration of beta-cell function *in vivo*.

(4) To modulate the regenerative niche and augment recovery of beta-cell function. Human mesenchymal progenitor cells will be engineered by retroviral transduction or siRNA technologies to over- or under-express key mediators of beta cell regeneration (such as GLP-1 or HGF), in order to augment the recovery of beta-cell function after transplantation *in vivo*.

Hypotheses. (1) Transplanted myeloid progeny of hematopoietic CFC will recruit to peri-islet regions and initiate endogenous beta-cell regeneration via paracrine mechanisms. **(2)** Peri-islet secretion of developmental and endocrine-acting cytokines will induce beta-cell proliferation, increase insulin

production, and accelerate the recovery from hyperglycemia. **(3)** Co-delivery of supportive mesenchymal-stromal cells will reduce local inflammation and enhance survival of regenerating islets.

Relevance. We will identify the cellular constituents and paracrine pathways that support endogenous beta-cell regeneration. These studies will provide pre-clinical justification for the development of novel cellular therapies to regenerate functional beta cell mass during the treatment of diabetes.

Cellular progenitors and molecular mediators of functional vascular regeneration.

Rationale

Rapid revascularization of diseased or ischemic organs is critical to all regenerative processes, allowing the delivery of circulating cells that formulate a regenerative niche. We have previously established that transplanted human bone marrow (BM) stem cells induce functional revascularization in immune deficient mice with critical limb ischemia (Capoccia et al, (2009) *Blood*). Transplanted purified progenitor cells with hematopoietic and mesenchymal phenotypes engrafted in ischemic regions, and induced mouse capillary formation and recovery of perfusion. This concept termed *stem cell-mediated vascular regeneration* has emerged as a central process during tissue repair after injury.

Background

Human BM and umbilical cord blood represent easily procured sources of progenitor cells from hematopoietic, endothelial, and mesenchymal stromal lineages that potentially modulate angiogenesis. Using high aldehyde dehydrogenase (ALDH) activity, an enzyme involved in the protection of cells from oxidative insult, we have identified a novel population of mixed-lineage progenitor cells based on a conserved stem cell function. High ALDH-activity isolates hematopoietic progenitor cells that reconstitute human hematopoiesis in immune-deficient mice. High ALDH-expression also enriched for expandable colony forming cells with endothelial and mesenchymal functions, rare regenerative cell types difficult to procure using typical stem cell surface markers. Intravenously transplanted ALDH^{hi} cells exhibit widespread tissue infiltration by non-hematopoietic cells, home efficiently ischemic tissues, and augment the endogenous revascularization of ischemic muscle tissue. Thus, cell selection using high ALDH-activity isolates multiple expandable progenitor lineages that potentially direct vessel formation through conserved paracrine mechanisms. *The specific progenitor lineages recruited to ischemic areas, the molecules secreted by regenerative cells, and the signaling pathways engaged in responding endothelial cells (EC) are currently unknown.*

Hypotheses

- (1) Hypoxia-expanded progenitor cells of distinct lineages will secrete pro-angiogenic cytokines that promote vascular EC survival, proliferation, and tubule formation *in vitro*.
- (2) Transplanted progenitors cells will recruit to ischemic regions and generate a pro-angiogenic microenvironment to potentiate endogenous revascularization *in vivo*.
- (3) Mesenchymal progenitor cells will act as pericytes to organize blood vessel assembly and stabilize perfused neovessels through the local secretion of pro-angiogenic cytokines.

Objectives

- (1) To characterize the hematopoietic, endothelial, and mesenchymal progenitor functions of expanded under normoxic versus hypoxic culture.
- (2) To characterize molecules secreted by human hematopoietic, endothelial, or mesenchymal progenitor cells that support EC survival, proliferation, and tubule formation *in vitro*.
- (3) To document the engraftment of transplanted cells in the ischemic region, and to characterize the purified progenitor cells blood vessel assembly *in vivo*.
- (4) To characterize the molecular pathways that blood vessel formation *in vivo*.

Relevance

Using unique *in vitro* co-culture systems and progenitor cell transplantation into novel immune-deficient mice with tissue ischemia, we will identify human cells that augment blood vessel formation, and functionally characterize molecular pathways that co-ordinate endogenous vascular regeneration. We propose that the cellular mechanisms that govern vascular repair in ischemic muscle will be conserved in other tissues such as damaged myocardium after infarct, or neural tissue after stroke. *Ultimately, this research will contribute to the development of novel drug and/or cellular therapies to treat ischemic tissue dysfunction in patients with diabetes, heart disease, and stroke.*



Product Description

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Cell Biology

ATCC® Number:	CRL-7522™	Order this Item	Price:	\$417.00
Designations:	Hs 792(C).M			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Homo sapiens</i> (human)			
Morphology:	fibroblast			
Source:	Tissue: muscle; connective and soft tissue Disease: normal Cell Type: fibroblast			
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.			
Cytogenetic Analysis:	modal number = 46; range = 41 to 46			
Age:	29 years			
Gender:	female			
Ethnicity:	Caucasian			
Comments:	Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC. We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description.			
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Atmosphere: air, 95%; carbon dioxide (CO ₂), 5% Temperature: 37.0°C			

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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 1:2 to 1:3 is recommended

Medium Renewal: Every 2 to 3 days

Preservation:

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

Related Products:

Storage temperature: liquid nitrogen vapor phase

Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2002](#)
derived from same individual: ATCC CRL-7520
recommended serum: [ATCC 30-2020](#)
derived from same individual: ATCC CRL-7521
derived from same individual: [ATCC CRL-7522](#)

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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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Preservation: culture medium 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2002](#)
recommended serum: [ATCC 30-2020](#)

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Product Description

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Cell Biology

ATCC® Number:	CRL-7449™	Order this Item	Price:	\$417.00
Designations:	Hs 707(B).Ep			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Homo sapiens</i> (human)			
Morphology:	fibroblast			
Source:	Organ: skin Tissue: epidermis 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.			
Cytogenetic Analysis:	modal number = 46; range = 45 to 46			
Age:	13 years			
Gender:	male			
Ethnicity:	Caucasian			
Comments:	The line was established from apparently normal skin tissue from a patient who had osteogenic sarcoma. Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC. We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description.			
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C			
Subculturing:	Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended Medium Renewal: Every 2 to 3 days Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.			

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Preservation: culture medium 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC [30-2002](#)
recommended serum: ATCC [30-2020](#)

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Cell Biology

ATCC® Number: **HTB-26™** [Order this Item](#)

Designations: MDA-MB-231

Depositors: R Cailleau

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Price: **\$256.00**

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Source: **Organ:** mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

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: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Yes

DNA Profile (STR): Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
TH01: 7,9,3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Gender: female

Ethnicity: Caucasian

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. 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, 100%
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 without CO₂.

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

Medium Renewal: 2 to 3 times per week

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

Related Products: **Storage temperature:** liquid nitrogen vapor phase
purified DNA:ATCC 45518
purified DNA:ATCC 45519
purified DNA:ATCC HTB-26D
purified RNA:ATCC HTB-26R
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008

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ASSURANCES & APPROVALS: (TO BE COMPLETED BY GRADUATE STUDENT)

All research proposals involving human and/or animal subjects, biohazardous or radioactive materials must be approved by a Western ethics, animal-care, or biohazards/radioactive review committee.

Indicate with an X on the chart below the current status of the approvals required for this proposal.
If approval has already been granted, an electronic copy of the approval notice must accompany this application.

		STATUS				
HUMAN SUBJECTS ETHICS APPROVAL	No human subjects	Approval granted	X	Pending		To be sought
		Authorization #: 12934				
ANIMAL SUBJECTS APPROVAL	No animal subjects	Approval granted	X	Pending		To be sought
		Authorization #: 2006-126-12				
BIOHAZARDOUS MATERIALS CLEARANCE	No biohazardous materials	Approval Granted	X	Pending		To be sought
		Authorization #: BIO-RRI-0029 Pending renewal				
RADIOACTIVE MATERIALS CLEARANCE	No radioactive materials	Approval granted	X	Pending		To be sought
		Authorization #: 2352-RRI-208				



Date: 09/30/2008

Dear Dr. Hess

An ANIMAL NUMBER ADJUSTMENT to your "Animal Use Protocol" 02006-126-12 entitled **Characterization of the Angiogenic Potential of Aldehyde Dehydrogenase Expressing Stem Cells from Human Bone Marrow** has been approved.

These strains and animal numbers are approved from 06/12/2008 until the protocol full expiry date 12/31/2010

SPECIES	STRAIN &/or OTHER SPECIES DETAIL	AGE or WEIGHT	SEX	ANIMAL NUMBER
Mouse	NOD SCID B2Mnull, bred at RBF	used at 6-12 weeks	V.F	750
Mouse	NOD SCID VPS VII, bred at RBF	used at 6-12 weeks	V.F	425
Mouse	NOD SCID bred at RBF	used at 6-12 weeks	Female	250
Mouse	NOD SCID IL2Rgamma null bred at RBF	used at 6-12 weeks	Female	250

s.c. Approval Letter - D. Hess, K. Lovic, M. Pickering

The University of Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, London, Ontario, CANADA - N6A 5C1
PH: 519-663-2111 ext. 86370 / FL: 519-661-2028 / www.uwo.ca/animal

2006-126 ANAF Approval Letter.pdf

July 22, 2008

Dear Dr. Hess:

Please note your biosafety approval number listed above. This number is very useful to you as a researcher working with biohazards. It is a requirement for your research grants, purchasing of biohazardous materials and Level 2 inspections.

Research Grants:

- This number is required information for any research grants involving biohazards. Please provide this number to Research Services when requested.

Purchasing Materials:

- This number must be included on purchase orders for Level 1 or Level 2 biohazards. When you order biohazardous material, use the on-line purchase ordering system (www.uwo.ca/finance/people/). In the "Comments to Purchasing" tab, include your name as the Researcher and your biosafety approval number.

Annual Inspections:

- If you have a Level 2 laboratory on campus, you are inspected every year. This is your permit number to allow you to work with Level 2 biohazards.

To maintain your Biosafety Approval, you need to:

- Ensure that you update your Biohazardous Agents Registry Form at least every three years, or when there are changes to the biohazards you are working with.
- Ensure that the people working in your laboratory are trained in Biosafety.
- Ensure that your laboratory follows the University of Western Ontario Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories.
- For more information, please see: www.uwo.ca/humanresources/biosafety.

Please let me know if you have questions or comments.

Regards,



Jennifer Stanley
Biosafety Coordinator for Western
Stevenson Lawson Building Room 295G
Phone: 519-661-2111 X31135
Fax: 519-661-3420



Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. D.A. Hess

Review Number: 12934

Revision Number: 1

Review Date: February 28, 2008

Review Level: Expedited

Protocol Title: Transplantation of human stem cells for the induction of angiogenesis and the regeneration of beta-cell function

Department and Institution: Vascular Biology, Robarts Research Institute

Sponsor: JUVENILE DIABETES RESEARCH FOUNDATION

Ethics Approval Date: February 28, 2008

Expiry Date: October 31, 2009

Documents Reviewed and Approved: revised study end date

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. John W. McDonald

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cc: ORE File
LHRI