Modification Form for Permit BIO-RRI-0029

Permit Holder: David Hess

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

Jennifer Hughes-Large

~~Paige Thompson~~

David Putman

Heather Broughton

Gillian Bell

~~Debra Robson~~

Additional Personnel
(Please list additional personnel here)

Ayesh Seneviratne

Eric Leci

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms		
Approved Primary and Established Cells	[Primary] (Human): Bone marrow, umbilical cord blood. (Mouse): Bone marrow. [Established]) (Human): MCF-7, Fibroblasts HS792 (c).M, skin fibroblast HS707 (B). Ep. (Rodent): MDA-MB-231, Beta-TC-6: mouse	[Primary] - Term & Pre-term Placental (Human) derived mesenchymal stromal Cells (MSC) - Human Pancreas derived MSC [Established] (Human) - Panc-1 - Human Umbilical vein endothelial cell (HUVEC) - Human Microvasculature endothelial cell (HMVEC)
Approved Use of Human Source Material	Human blood (whole) or other body fluid, Unpreserved human organ or tissues: human bone marrow.	
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] - pGIPZ, pIRES2-EGFP-Cox2, pIRES2-EGFP	
Approved Use of Animals	(mice): NOD/SCID, NoD/SCID K-2R null, NoD/SCID/MPSVI	
Approved Biological Toxin(s)		

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

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

Signature of Permit Holder: David Hess

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

Date of Last Biohazardous Agents Registry Form: Nov 24, 2009

Date of Last Modification (if applicable): Feb 18, 2011

BioSafety Officer(s): _____

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

Use, storage, and disposal of:

Term and Pre-Term Human Placental Derived Mesenchymal Stromal Cells (MSC):
These cells will be cultured and expanded for use in our diabetic mice associated with protocol 2006-122-12 as part of a collaboration with Dr. Han. Dr. Han staff will store them in their lab. All cells are handled as level 2 and extra cells will be bleached. Mice transplanted with these cells will also be handled with level 2 precautions.

Pancreas Derived MSC:
These cells will be cultured and expanded for use in our diabetic mice associated with protocol 2006-122-12. They will be stored in our liquid nitrogen and handled with level 2 procedures. All extra cells will be bleached. Mice transplanted with these cells will also be handled as level 2.

Panc-1:
These cells will be cultured and expanded for use in our diabetic mice associated with protocol 2006-122-12. We will also use these cells in tissue culture for specific co-culture experiments. They will be stored in our liquid nitrogen and handled with level 2 procedures. All extra cells will be bleached. Mice transplanted with these cells will also be handled as level 2.

Human Umbilical vein Endothelial Cells (HUVEC) AND Human Microvascular Endothelial cell (HMVEC):
These cells will be cultured and expanded for use in our hind limb ischemia mouse model associated with protocol 2006-126-12. We also will use these cells in tissue culture for co-culture and matrigel assays. They will be stored in our liquid nitrogen and handled with level 2 procedures. All extra cells will be bleached. Mice transplanted with these cells will also be handled as level 2.

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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Cell Biology

ATCC® Number: CRL-1469™ (Order this Item) **Price:** $279.00

Designations:	PANC-1
Depositors:	M Lieber
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** pancreas **Tissue:** duct **Disease:** epithelioid carcinoma
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 (Roche Transfection Reagents)
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 11 D16S539: 11 D5S818: 11,13 D7S820: 8,10 THO1: 7,8 TPOX: 8,11 vWA: 15
Cytogenetic Analysis:	Chromosome studies indicate a modal number of 63 with 3 distinct marker chromosomes and a small ring chromosome. This is a hypertriploid human cell line. The modal chromosome number was 61, occurring in 32% of cells., However, cells with 63 chromosomes also occurred at a high frequency (22%). The rate of cells with higher ploidies was 8.5%.
Isoenzymes:	G6PD, B
Age:	56 years

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Product Information Sheet

Gender:	male
Ethnicity:	Caucasian
Comments:	Growth is inhibited by 1 unit/ml L-asparaginase. The cells will grow in soft agar.
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 **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.53mM 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.
Preservation:	culture medium 95%; DMSO, 5%
Doubling Time:	52 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	22850: Lieber M, et al. Establishment of a continuous tumor-cell line (panc-1) from a human carcinoma of the exocrine pancreas. Int. J. Cancer 15: 741-747, 1975. PubMed: 1140870 22859: Wu MC, et al. Mechanism of sensitivity of cultured pancreatic carcinoma to asparaginase. Int. J. Cancer 22: 728-733, 1978. PubMed: 363626 23079: Lan MS, et al. Polypeptide core of a human pancreatic tumor mucin antigen. Cancer Res. 50: 2997-3001, 1990. PubMed: 2334903

Return to Top

Back to my Search

Lonza Walkersville, Inc.
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biotechserv@lonza.com
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Walkersville, MD 21793-0127 USA

Terms and Conditions

Definitions
These Terms and Conditions apply to Lonza Rockland and Lonza Walkersville, herein referred to as "Seller."

Warranty
Due to the various factors affecting research test results, Seller warrants only and not for any particular purpose of the purchases, that all products sold will perform according to established product specifications. Products are sold with the understanding that the purchaser will determine if the product is suitable for his or her application. We will replace any product, free of charge, that does not meet our established product release specifications. Seller shall not be liable for any damages or injury to persons or property arising from the purchase or use of the product. In no event shall Seller's liability exceed the purchase price paid by the buyer. In addition, we are not liable for the product after the product expiration date or for a product that has been misused or has become unusable due to improper storage or handling by purchaser.

Seller guarantees the performance of Clonetics® and Poietics® cells up to two years from purchase only if appropriate Clonetics® or Poietics® media and reagents are used exclusively, and the recommended storage and use protocols are followed. Cell and media performance is not guaranteed if any modifications are made to such cell systems.

Product Use
Clonetics® and Poietics® cell products are intended for research purposes only and the buyer has no rights to transfer the products, components, or materials made using these products, or, use these products for Commercial Purposes. Commercial Purposes includes 1) use of the products or their components in manufacturing; 2) use of the products or their data components to provide a service, information, or data; 3) use of the products or their components for therapeutic or diagnostic purposes; 4) resale of the products or their components.

Safety Statement
THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.
WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Technical Services.

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8830 Biggs Ford Road
Walkersville, MD 21793 8415
Tel (301) 898 7025
Fax (301) 845 4024

Lonza

CERTIFICATE OF ANALYSIS

Product Code: CC-2543
Product: HMVEC-d Ad-Dermal MV Endo Cells,EGM-2MV, cryo amp

Lot Number: 7F3852
Manufacture Date: 10-Aug-2007

TEST (Method)	SPECIFICATIONS Min.	Max.	Results
Tissue Acquisition Number	***	***	15968
DONOR CHARACTERISTICS			
Age	***	***	42 Y
Sex	***	***	FEMALE
Race	***	***	B
VIRUS TESTING			
HIV Test	***	***	Not detected
HBV Test	***	***	Not detected
HCV Test	***	***	Not detected
MICROBIAL TESTING			
Sterility - Amp	***	***	Negative
Direct Plating (Mycoplasma)	***	***	Negative
CELL PERFORMANCE TESTING			
Cell Passage Frozen			3
Viability-Tryp.Blue Exclusion	> = 70%	***	89 %
Cell Count (Cells/ml)	> = 500,000	***	686000
Total Population Doublings	> = 15	***	15
Seeding Efficiency	> = 20%	***	70 %
Doubling Time (hours)	15	48	27
Alpha Actin Expression	Negative	***	Pass

This lot has been isolated from human tissue obtained under "informed consent." Details concerning the use of our cell and media products can be downloaded from our website at www.lonza.com.

In addition to the specifications listed above, the following are guaranteed for all lots of this product using Lonza's Clonetics(TM) and Poietics(TM) Media, Reagents, and Protocols: Factor VIII Positive, Acetylated LDL Uptake Positive.

This lot has been reviewed by Quality Assurance in compliance with requirements of Lonza's Quality System.
This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.

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Walkersville, MD 21793 0107
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Lonza

Printed on, 11-Nov-2008 17:22 rec'd Nov. 12-08

CERTIFICATE OF ANALYSIS

Product Code: C2519A
Product: HUVEC-Umbil Vein, Pooled Cells,EGM-2, cryo amp

Lot Number: 8F3275
Manufacture Date: 12-Mar-2008

TEST (Method)	SPECIFICATIONS Min.	Max.	Results
Tissue Acquisition Number	***	***	P767
Donor Screen Information:			
Age	***	***	NB
Sex	***	***	MALE/FEMALE MIXED
Race	***	***	B,C,C
Cell Type	***	***	HUVEC POOL
QC Evaluation Medium			EGM 2
Date of Cryopreservation	***	***	12.03.2008
Cell Strain Calculations:			
Cell Passage			1
Viability-Tryp.Blue Exclusion	> = 70%	****	74.00 %
Cell Count (Cells/ml)	> = 500,000	****	600000
Total Population Doublings	> = 15	****	15
Seeding Efficiency	> = 20%	*****	65 %
Doubling Time (hours)	12	48	17.00
Sterility - Amp	****	*****	Negative
Direct Plating (Mycoplasma)	****	****	Negative
Virus Testing:			
HBV Test	*****	*****	Not detected
HIV Test	****	****	Not detected
HCV Test	****	*****	Not detected

This lot has been isolated from human tissue obtained under "informed consent". Details concerning the use of our cell and media products can be downloaded from our website at www.lonza.com.

This lot has been reviewed by Quality Assurance in compliance with requirements of Lonza's Quality System.
This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.

Modification Form for Permit BIO-RRI-0029

Permit Holder: David Hess

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

~~Paige Thompson~~

David Putman

Heather Broughton

Gillian Bell

Debra Robson

Additional Personnel
(Please list additional personnel here)

Jennifer Hughes-Large

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms		
Approved Primary and Established Cells	[Primary] (Human): Bone marrow, umbilical cord blood. (Mouse): Bone marrow. [Established]) (Human): Fibroblasts HS792 (c).M, skin fibroblast HS707 (B). Ep. (Rodent): MDA-MB-231, Beta-TC-6: mouse	MCF-7
Approved Use of Human Source Material	Human blood (whole) or other body fluid, Unpreserved human organ or tissues: human bone marrow.	
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] - pGIPZ	plasmid - pIRES2-EGFP-COX2 - pIRES2-EGFP
Approved Use of Animals	(mice): NOD/SCID, NoD/SCID K-2R null, NoD/SCID/MPSVI	
Approved Biological Toxin(s)		

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** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

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Signature of Permit Holder: David Hess

Current Classification: 2 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Nov 24, 2009

Date of Last Modification (if applicable): Jan 15, 2010

BioSafety Officer(s): JStanley Feb 17/11, Ronald Dosen Feb.08/11

Chair, Biohazards Subcommittee: [signature] **Date:** Feb 18 2011

Brief description

We have recently started a collaboration with the Lala Lab. The Lala Lab will be responsible for all culture related tasks for the MCF cell line. They will hand us the cells to be transplanted into our mice via the tail vein. The mice will sit for 4 – 8 weeks post injection and will then be sacrificed for tissue analysis.

Product Information Sheet for ATCC® HTB-22™

Cell Line Designation: MCF-7
ATCC® Catalog No. HTB-22™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 69 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Doubling time: about 29 hours
Growth Properties: adherent
Oncogene: wnt7h +
Antigens Expressed: Blood Type O; Rh+
Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
TH01: 6
TPOX: 9,12
vWA: 14,15

Depositor: C.M. McGrath
Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.
Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Karyology: modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%.
There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases.
No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA from this line is available as ATCC® HTB-22D™ (10µg)
Total RNA from this line is available as ATCC® HTB-22R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at

American Type Culture Collection
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Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cell pellet to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Note: Present batches of MCF7 cells are exhibiting the following growth pattern:
The cells usually attach as three-dimensional clusters and eventually grow to a 80-90% confluent monolayer. However, we are finding that most of the clusters remain in suspension until after the 2nd subculture.
After first subculture all the cells will not attach. There will be clusters in suspension. Break up the clusters the best you can by gently pipetting with a small bore pipette (5 ml or smaller). After a few days incubation, the cells should reattach as three-dimensional islands (there will be some clusters that do not reattach). Growth will eventually spread out from the islands and the culture should, after the second subculture, flatten and become 70-80% confluent.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.

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 with 5 to 10 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. Transfer the cell suspension to the centrifuge tube with the medium and cells from Step #1 and spin at approximately 125 xg for 5 to10 minutes. Discard the supernatnant.

6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Maintain cultures at a cell concentration between 2x10(4) and 2 x 10(5) cells/cm2.
   Subcultivation Ratio: 1:3 to 1:6.

7. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- 0.01mg/ml bovine insulin
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) or ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog at www.atcc.org)

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Takahashi K and Suzuki K. **Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7** cells. Int. J. Cancer 55: 453-458, 1993 PubMed: 93388025

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Landers JE et al. **Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein.** Cancer Res. 57: 3562-3568, 1997 PubMed: 97413643

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Bacus SS et al. **Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen.** Mol. Carcinog. 3: 350-362, 1990 PubMed: 91119659

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines.** 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

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05/09

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Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2, BP-4, BP-5
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:	estrogen receptor, expressed
Antigen Expression:	Blood Type O; Rh+
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15
Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Isoenzymes:	AK-1: 1 ES-D: 1-2 G6PD: B GLO-I: 1-2 PGM1: 1-2 PGM3: 1
Age:	69 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10%.

Related Links

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Material Transfer Agreement

Technical Support

Related Cell Culture Products

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Return to Top

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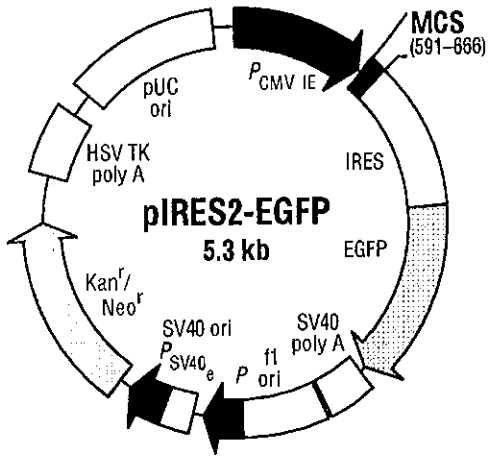
Parental vector

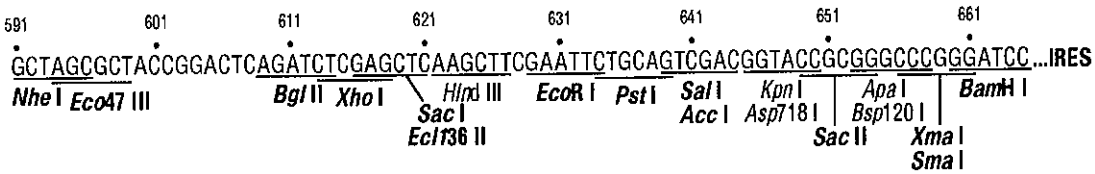
Vector Information

pIRES2-EGFP Vector Information

PT3267-5

Catalog #6029-1





Restriction Map and Multiple Cloning Site (MCS) of pIRES2-EGFP Vector. Unique restriction sites are in bold. Note that the *Eco*47 III site has not been confirmed in the final construct.

Description:

pIRES2-EGFP contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and the enhanced green fluorescent protein (EGFP) coding region. This permits both the gene of interest (cloned into the MCS) and the EGFP gene to be translated from a single bicistronic mRNA. pIRES2-EGFP is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing EGFP and the protein of interest. This vector can also be used to express EGFP alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

EGFP is a red-shifted variant of wild-type GFP (3–5) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) EGFP encodes the GFPmut1 variant (6) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (7). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (8) to further increase the translation efficiency in eukaryotic cells. The MCS in pIRES2-EGFP is between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the bicistronic mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pIRES2-EGFP backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. pIRES2-EGFP replaces (but is not derived from) the pIRES-EGFP Vector previously sold by BD Biosciences Clontech. pIRES2-EGFP is functionally similarly to pIRES-EGFP; however, pIRES2-EGFP gives brighter EGFP fluorescence than the older vector. Note that the *Xba* I site at position

(PR29951; published 03 October 2002)

Clontech

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A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

1987 is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*$^-$ host and make fresh DNA.

Use:
Genes inserted into the MCS should include the initiating ATG codon. pIRES2-EGFP and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

Location of features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MCS: 591–665
- IRES sequence: 666–1250
- Enhanced green fluorescent protein (EGFP) gene
  Kozak consensus translation initiation site: 1247–1257
  Start codon (ATG): 1254–1256; Stop codon: 1971–1973
  Insertion of Val at position 2: 1257–1259
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 1446–1451
  His-231 to Leu mutation (A→T): 1948
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2127–2132 & 2156–2161; mRNA 3' ends: 2165 & 2177
- f1 single-strand DNA origin: 2224–2679 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2741–2746; –10 region: 2764–2769
  Transcription start point: 2776
- SV40 origin of replication: 3020–3155
- SV40 early promoter/enhancer
  72-bp tandem repeats: 2853–2996; 21-bp repeats (3): 3000–3063
  Early promoter element: 3076–3082
- Kanamycin/neomycin resistance gene: 3204–3998
  G→A mutation to remove *Pst* I site: 3386; C→A (Arg to Ser) mutation to remove *Bss*H II site: 3732
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4234–4252
- pUC plasmid replication origin: 4583–5226

Propagation in *E. coli*
- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:
1. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**:477–483.
2. Jang, S. K., *et al.* (1990) *J. Virol.* **62**:2636–2643.
3. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
4. Yang, T. T., *et al.* (1996) *Nucleic Acids Res.* **24**:4592–4593.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Jackson, R. J., *et al.* (1990) *Trends Biochem.* **15**:477–483.
7. Jang, S. K., *et al.* (1988) *J. Virol.* **62**:2636–2643.
8. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947.
9. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

Notice to Purchaser

Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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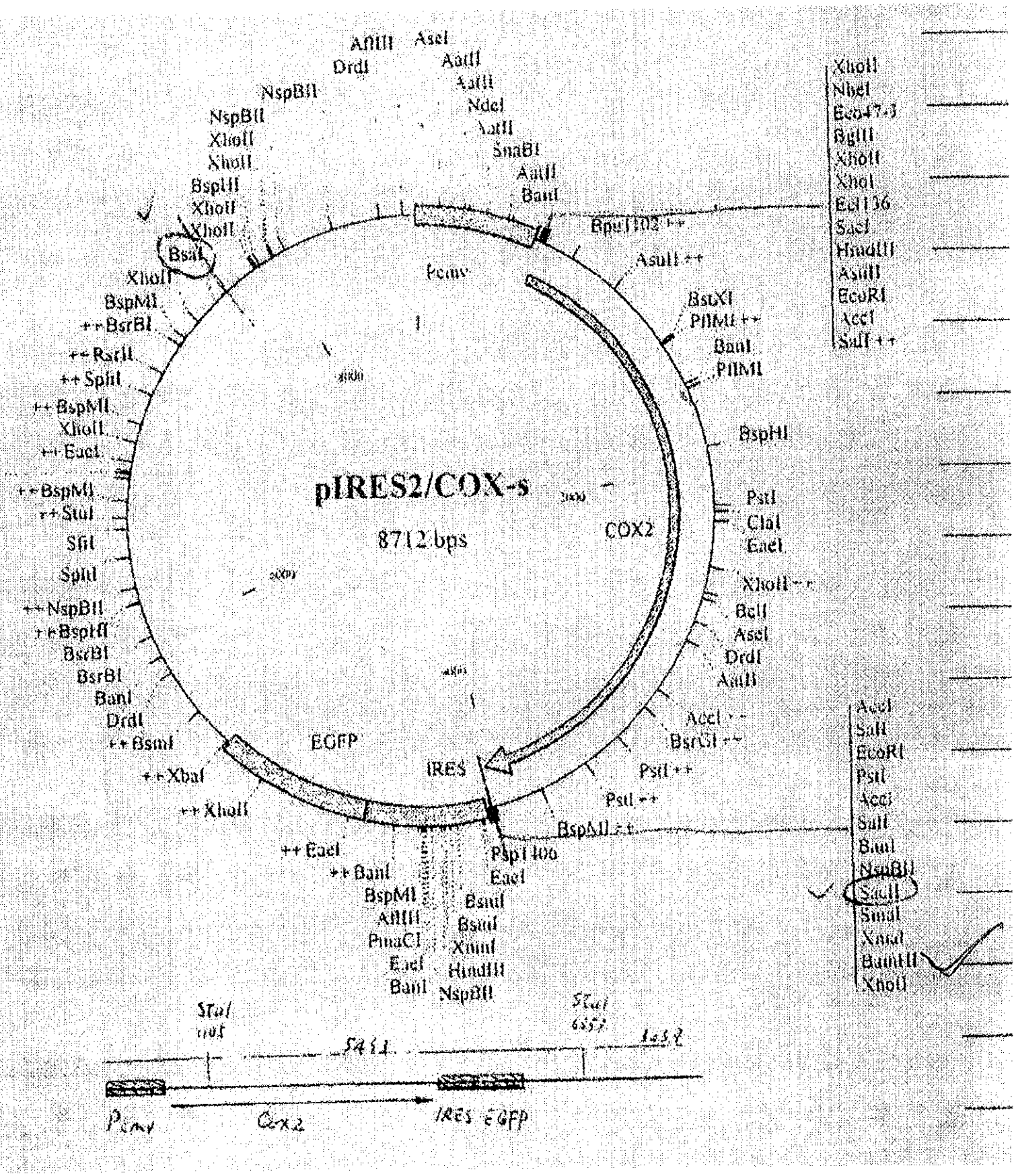
Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support. All companies and institutions purchasing Living Colors™ products will be included in a quarterly report to Aurora Biosciences, as required by the BD Biosciences Clontech/Aurora Biosciences license agreement.

Use of the IRES sequence is covered by U.S. Patent #4,937,190 and is limited to use solely for research purposes. Any other use of the IRES sequence requires a license from Wisconsin Alumni Research Foundation.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. BD Biosciences Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of BD Biosciences Clontech.

Expression Vector



Modification Form for Permit BIO-RRI-0029

Permit Holder: David Hess

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

Paige Thompson

David Putman

Heather Broughton

Gillian Bell

Debra Robson

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	[Primary] (Human): Bone marrow, umbilical cord blood. (Mouse): Bone marrow. [Established]) (Human): Fibroblasts HS792 (C)M, skin fibroblast HS707 (B). Ep. (Rodent): MDA-MB-231	Beta-TC-6 mouse beta cell line - level 1 cell line ℓ.
Approved Use of Human Source Material	Human blood (whole) or other body fluid, Unpreserved human organ or tissues: human bone marrow.	
Approved GMO	[Plasmid] - pGIPZ	
Approved use of Animals	(mice): NOD/SCID, NoD/SCID K-2R null, NoD/SCID/MPSVI ← Capital "O"	
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.*

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

Signature of Permit Holder: David Hess

Classification: 2

Date of Last Biohazardous Agents Registry Form: Nov 24, 2009

Date of Last Modification (if applicable): David Hess

BioSafety Officer(s): JL Tunley Jan 15, 2010

Chair, Biohazards Subcommittee: G.M. Fuller

Beta-TC-6 cell line will be used for *in vitro* analysis: characterization of cell surface markers and behaviour in cell culture as a control compared to bone marrow and cord blood derived cell lines.

Cell Biology

ATCC® Number:	**CRL-11506™** Order this Item	Price:	**$349.00**
Designations:	Beta-TC-6		
Depositors:	CytoTherapeutics, Inc.		
Biosafety Level:	2 [Cells contain SV40 viral DNA Sequences]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	Mus musculus, transgenic for SV40 large T antigen (mouse, transgenic for SV40 large T antigen)		
Morphology:	epithelial 		
Source:	**Organ:** pancreas **Disease:** insulinoma **Cell Type:** beta cell;		
Cellular Products:	insulin, glucagon and somatostatin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Comments:	The cell line was derived from a pancreatic tumor (insulinoma) arising in a transgenic mouse. The mouse carried the pseudogene construct composed of the SV40 early region controlled by the rat insulin II gene promotor. The cells contain abundant insulin and small amounts of glucagon and somatostatin. They secrete insulin in response to glucose. [46884]		
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: heat-inactivated fetal bovine serum to a final concentration of 15%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C		

Related Links ▸

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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.

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended

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

Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

46126: Laurance ME, et al. Glucose responsive insulin secreting beta-cell lines and method for producing same. US Patent 5,773,255 dated Jun 30 1998

References:

46884: Poitout V, et al. Morphological and functional characterization of beta TC-6 cells--an insulin-secreting cell line derived from transgenic mice. Diabetes 44: 306-313, 1995. PubMed: 7533732

46885: Poitout V, et al. Insulin-secreting cell lines: classification, characteristics and potential applications. Diabetes Metab. 22: 7-14, 1996. PubMed: 8697299

Return to Top

Ron Noseworthy

From: David Hess [dhess@robarts.ca]
Sent: January 12, 2010 1:52 PM
To: Ron Noseworthy
Subject: Re: Biohazard Modification

Absolutely Ron, these cell lines will not be infected with retrovirus or lentivirus vectors.

Dave Hess

>Hi Dr. Hess,
>
>Can you confirm that the cells on your most recent modification:
>HS707(B) and Beta-TC-6 will not be infected with Retro or lentivral
>vectors?
>
>Thanks
>
>Ron
>Ron Noseworthy MCIC CRSP
>Manager, Occupational Health and Safety Robarts Research Institute UWO,
>Schulich School of Medicine and Dentistry 100 Perth Drive, P.O. Box
>5015 London, Ontario N6A 5K8
>Phone: 519-663-5777 ext. 24125
>Fax: 519-931-5267
>

--
David A. Hess PhD

Assistant Professor, Department of Physiology and Pharmacology University of Western Ontario Scientist, Vascular Biology Group Krembil Centre for Stem Cell Biology Robarts Research Institute PO Box 5015, 100 Perth Drive, Room E4-19 London, Ontario, Canada, N6A 5K8
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email: dhess@robarts.ca

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 1998, 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: Roberts Research Institute/Physiology & Pharmacology
ADDRESS: 100 Perth Dr, London, ON, N6A 5K8
PHONE NUMBER: 519-663-5777 x 24152
EMERGENCY PHONE NUMBER(S): 519-645-2703
EMAIL: dhess@robarts.ca

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

*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 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# 86759 "~~Mechanotransduction~~ Cellular constituents and paracrine factors that mediate functional vascular regenerato

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 Lofgren - Technician
Gillian Bell - Graduate Student
David Putman - Graduate Student
Paige Thompson - Undergrad Student

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

1.1 Does your work involve the use of biological agents? O 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? O YES O 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
	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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	✓Yes O No	Human bone Marrow Human umbilical cord blood	Not applicable
Rodent	✓Yes O No	Mouse bone Marrow.	2006-120-12 2006-122-12
Non-human primate	O Yes ✗ No		
Other (specify)	O Yes ✗ 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	✓ Yes O No	Human Fibroblasts HS 71(C).M	- ATCC
		Human Skin Fibroblast HS 707(B).Ep	- ATCC
Rodent	O Yes ✓ No	Human breast Cancer MDA-MB-231	- ATCC
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ✓YES O 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 BM - University Hosp.	~~O~~ Yes O No ✓ Unknown	Not Applicable	O 1 ✓ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes ✓ No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)	Human Bone Marrow University Hospital	~~O~~ Yes ✓ No ✓ Unknown	Not Applicable	O 1 ✓ 2 O 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 O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
None - purified DNA only	pGIPZ	Open Biosystems	-shRNA Nodal -shRNA Control	-expected inhibition of Nodal expression =tumor cell growth and angiogenesis control - no change

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

4.3 Will genetic modification(s) involving viral vectors be made? O 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 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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used NOD/SCID, NOD/SCID IL-2Rγnull, NOD/SCID/MPSVII

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 O YES, specify: _____ ☑ NO

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

_____

_____

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

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø 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_____

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? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

*** 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? O 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? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O 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? O YES O NO
If NO, please forward the permit to he 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? O 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? O YES O NO

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 _____

*** 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. O 1 Ø 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
Ø YES, permit # if on-campus Bio-RL1-0029
O NO, please certify
O 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: [signature]
Date: 24 Nov. 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: November 23, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: [signature]
Date: NOV 23/09

Approval Number: Bio-RL1-0029 Expiry Date (3 years from Approval): NOV 23, 2012

Special Conditions of Approval:

-------- Original Message --------
Subject: Fw: Fwd: Re: pGIPZ plasmid
Date: Mon, 23 Nov 2009 08:29:41 -0500
From: Ron Noseworthy <rnoseworthy@robarts.ca>
To:jstanle2@uwo.ca

Hi Jennifer

Here the response from Dr. Hess regarding the plasmid.

Ron

----- Original Message -----
From: David Hess <dhess@robarts.ca>
To: Ron Noseworthy
Sent: Sun Nov 22 10:48:51 2009
Subject: Fwd: Re: pGIPZ plasmid

Hi Ron,

Please find below Lynne Postovit's response to our inquiry aboout the pZGIP plasmid system she uses in her lab for the Nodal shRNA knockdown.

Please let me know if this is sufficient for the Biohazzard Committee.

Thanks

David

>Date: Fri, 20 Nov 2009 16:28:59 -0500
>From: "Lynne Postovit" <Lynne.Postovit@schulich.uwo.ca>
>To: "David Hess" <dhess@robarts.ca>
>Subject: Re: pGIPZ plasmid
>
>Hey Dave:
>
>We did not use a lentiviral transduction. We simply use the
>plasmid. The cells were transfected via traditional methods with
>arrestin.
>
>Thanks!
>
>Lynne

>
>Lynne-Marie Postovit, PhD
>Assistant Professor
>438 Medical Science Building
>Dept. of Anatomy & Cell Biology
>The Schulich School of Medicine
>University of Western Ontario
>London, Ontario
>Canada N6A 5C1
>Phone: (519) 661-2111 x 80524
>Fax: (519) 661-3936
>
>
>>>> David Hess <dhess@robarts.ca> 11/20/2009 2:54 PM >>>
>Hi Lynne,
>
>Our Biohazzard renewal was reviewed today by the committee and I have
>been asked to provide more information on the pGIPZ vector that you
>use to transfect the shRNA int6o the MDA-231 cells.
>
>They are concerned that this is a lentiviral transduction vector
>which would push our biohazzard level to the intrepid level 2+. I
>told our biohazzard representative that this is not a lentiviral
>transduction and just the pGIPZ plasmid is used. However he needs
>this in writing.
>
>Can you send me a short email explaining how you use the pGIPZ vector
>with these cells and state clearly that you in no way use a full
>lentiviral transduction?
>
>I will forward this email to Ron and we will be all cleared.
>
>Thanks
>
>Dave
>
>PS. Me and my grad student Dave are going to the grad club now. It
>would be great if you could join us! See you there!
>--
>David A. Hess PhD
>
>Assistant Professor, Department of Physiology and Pharmacology
>University of Western Ontario
>Scientist, Vascular Biology Group

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-2Rγ 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.***

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Growth Properties:	adherent		Material Transfer Agreement
Organism:	*Homo sapiens* (human)		Technical Support
Morphology:	fibroblast		Related Cell Culture Products
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 (CO2), 5% **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 1:2 to 1:3 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	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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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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Morphology:	fibroblast		Related Cell Culture Products
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.		

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

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Designations:	MDA-MB-231
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Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
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 THO1: 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.

Price: **$256.00**

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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 **Storage temperature:** liquid nitrogen vapor phase
Related Products:	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

References:

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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					

Western

Date: 09 30 2009

Dear Dr. Hess

An ANIMAL NUMBER ADJUSTMENT to your Animal Use Protocol #2006-126-12 entitled **Characterization of the Angiogenic Potential of Aldehyde Dehydrogenase Expressing Stem Cells from Human Bone Marrow** has been approved.

These animal numbers are approved from 09 12 2009 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	M F	750
Mouse	NOD SCID VPS VII bred at RBF	used at 6-12 weeks	M F	450
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

c.c Approval Letter - D. Hess, K. Lizzac, M. Pickering

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre London Ontario CANADA N6A 5C1
PH 519-661-2111 ext 86330 FL 519-661-2028 www.uwo.ca/animal

Western

Biosafety Approval Number: BIO-RRI-0029

Expiry Date: October 23, 2009

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 X81135
Fax: 519-661-3420

Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Western

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

Ethics Officer to Contact for Further Information

☐ Janice Sutherland (jsutherl@uwo.ca)	☑ Jennifer McEwen (jmcewen4@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)	☐ Denise Grafton (dgrafton@uwo.ca)

This is an official document. Please retain the original in your files.

cc: ORE File
LHRI