

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	<p>[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</p>	MCF-7
Approved Use of Human Source Material	<p>Human blood (whole) or other body fluid, Unpreserved human organ or tissues: human bone marrow.</p>	
Approved Genetic Modifications (Plasmids/Vectors)	<p>[Plasmid] - pGIPZ</p>	<p>plasmid - pIRES2-EGFP-cox2 - pIRES2-EGFP</p>
Approved Use of Animals	<p>(mice): NOD/SCID, NoD/SCID K-2R null, NoD/SCID/MPSVI</p>	
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: 1

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

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

BioSafety Officer(s): Ronald Noseworthy Feb. 09/11

Chair, Biohazards Subcommittee: _____ Date: _____

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
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

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 hypotriploidy 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

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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)

Sugarman BJ et al. **Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro.** *Science* 230: 943-945, 1985
PubMed: 86044518

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
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Brandes LJ and Hermonat MW. **Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol.** *Cancer Res.* 43: 2831-2835, 1983
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Zhu X et al. **Cell cycle-dependent modulation of telomerase activity in tumor cells.** *Proc. Natl. Acad. Sci. USA* 93: 6091-6095, 1996
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Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

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Product Information Sheet for ATCC® HTB-22™

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05/09

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Designations: MCF7
Depositors: CV McGrath
Biosafety Level: 1
Shipped: frozen
Medium & Serum: [See Propagation](#)
Growth Properties: adherent
Organism: *Homo sapiens* (human)
Morphology: epithelial

Related Links

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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 [MIA](#) 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
 TH01: 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, 8
 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. 30-2902. 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%.

[Return to Top](#)**Neisseria meningitidis**

ATCC 49619 is a reference strain of *Neisseria meningitidis* serotype 4. It is not intended for use in humans.

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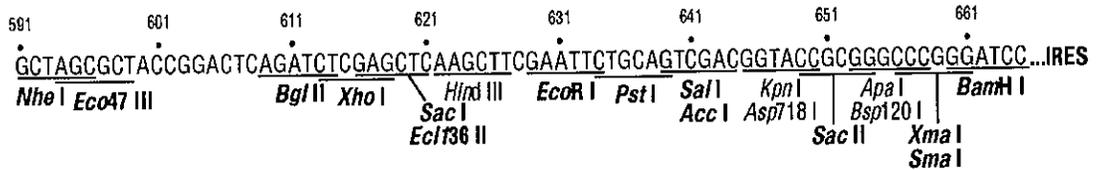
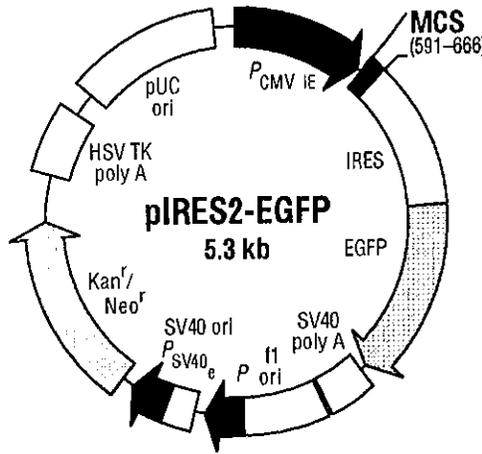
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pIRES2-EGFP Vector Information

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 *Eco47 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 similar 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)



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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: DH5 α , 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 μ g/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.
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7. Jang, S. K., *et al.* (1988) *J. Virol.* **62**:2636–2643.
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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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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

(c) ← [Primary] (Human): Bone marrow, umbilical
cord blood. (Mouse): Bone marrow.
[Established] (Human): Fibroblasts HS792
© M, skin fibroblast HS707 (B). Ep. (Rodent):
MDA-MB-231

Beta-TC-6% mouse
beta cell line

- Level
1
cell
line
pl.

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): *Stanley* Jan 15, 2010

Chair, Biohazards Subcommittee: *G.M. Keller*

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		Related Links ▶	
Depositors:	CytoTherapeutics, Inc.		NCBI	
<u>Biosafety Level:</u>	2 [Cells contain SV40 viral DNA Sequences]		Entrez	
Shipped:	frozen		Search	
Medium & Serum:	See Propagation		Cell	
Growth Properties:	adherent		Micrograph	
Organism:	Mus musculus, transgenic for SV40 large T antigen (mouse, transgenic for SV40 large T antigen) epithelial		Make a	
Morphology:	 Organ: pancreas		Deposit	
Source:	Disease: insulinoma Cell Type: beta cell;		Frequently	
Cellular Products:	insulin, glucagon and somatostatin		Asked	
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.		Questions	
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]		Material	
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		Transfer	
			Agreement	
			Technical	
			Support	
			Related Cell	
			Culture	
			Products	

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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

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

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

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

References:

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 lentiviral

>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

Office: 519-663-5777 ext. 24152

Lab: 519-663-5777 ext. 24415

Fax: 519-663-3789

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 81 135 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
 SIGNATURE
 DEPARTMENT
 ADDRESS
 PHONE NUMBER
 EMERGENCY PHONE NUMBER(S)
 EMAIL

David Hess
David Hess
Robarts Research Institute/Physiology & Pharmacology
100 Perth St, London, ON, N6A 5K8
519-663-5777 x 24152
519-645-2703
dhess@robarts.ca

Location of experimental work to be carried out: Building(s) Robarts Room(s) 4250, 4244A
 *For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for Robarts Experimental 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 "Maturation of 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
Grillian 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	Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Human umbilical cord blood Mouse bone Marrow.	2006-120-12 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		

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 720, M	- ATCC
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No	Human Skin Fibroblast HS 107 (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. Sue's Birthing Center Uch - University Hospital	<input checked="" type="radio"/> Yes <input type="radio"/> No <input 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 type="radio"/> No <input 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	- gene inhibition of Nodal expression = control - no change + emerging

* 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-2Ry null, NOD/SCID/MP5VII

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 *Carroll Hesse*

*** 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 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus BIO-RR1-0029
 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 Hess 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: EM K. J. J.
Date: 2nd Nov, 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: Paul Westwood
Date: November 23, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: Alanley
Date: Nov 23/09

Approval Number: BIO-RR1-0029 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

This message is intended only for the individual or entity to which it is addressed and may contain information that is privileged, confidential and exempt from disclosure under applicable law. If you are not the intended recipient, any dissemination, distribution or copying of this communication is prohibited. If you have received this communication in error, please notify the sender and delete the original. Thank you.

----- 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 about the pZGIP plasmid system she uses in her lab for the Nodal shRNA knockdown.

Please let me know if this is sufficient for the Biohazard 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 <dhes@robarts.ca> 11/20/2009 2:54 PM >>>

>Hi Lynne,

>
>Our Biohazard 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 into the MDA-231 cells.

>
>They are concerned that this is a lentiviral transduction vector
>which would push our biohazard level to the intrepid level 2+. I
>told our biohazard 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-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 progenitor 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

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

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Cell Biology

ATCC® Number:	CRL-7522™	Order this Item	Price:	\$417.00
Designations:	Hs 792(C).M		Related Links ▶	
Biosafety Level:	1		NCBI Entrez Search	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (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 (CO ₂), 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

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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Preservation: culture medium 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 10-2002](#)
recommended serum: [ATCC 10-2020](#)

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

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Cell Biology

ATCC® Number:	CRL-7449™	Order this Item	Price:	\$417.00
Designations:	Hs 707(B).Ep		Related Links ▶	
Biosafety Level:	1		NCBI Entrez Search	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human)		Technical Support	
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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Product Description

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Cell Biology

ATCC® Number:	HTB-26™ Order this Item	Price:	\$256.00
Designations:	MDA-MB-231	Related Links ▶	
Depositors:	R Cailleau	NCBI Entrez Search	
Biosafety Level:	1	Cell Micrograph	
Shipped:	frozen	Make a Deposit	
Medium & Serum:	See Propagation	Frequently Asked Questions	
Growth Properties:	adherent	Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human)	Technical Support	
Morphology:	epithelial	Related Cell Culture Products	
Source:	<p>Organ: mammary gland; breast Disease: adenocarcinoma Derived from metastatic site: pleural effusion Cell Type: epithelial</p>		
Permits/Forms:	<p>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.</p>		
Applications:	<p>transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)</p>		
Receptors:	<p>epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed</p>		
Tumorigenic:	<p>Yes</p>		
DNA Profile (STR):	<p>Amelogenin: X CSF1PO: 12,13 D13S317: 13 D16S539: 12 D5S818: 12 D7S820: 8,9 TH01: 7,9,3 TPOX: 8,9 vWA: 15,18</p>		
Cytogenetic Analysis:	<p>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.</p>		

Isoenzymes: AX-1, 1
ES-O, 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

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					



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 proposed animal numbers are approved from 06/12/2008 until the protocol full expiry date 12/21/2010.

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	500
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

cc: Approval Letter - D. Hess, K. Linnik, 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. 39370 | FL: 519-663-2028 | www.uwo.ca/animal

2006-126 ANAF Approval Letter.pdf

Printed for David Hess <dhes@robarts.ca>



Biosafety Approval Number: BIO-RR1-0029

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 Stanby
Biosafety Coordinator for Western
Stevenson Lawson Building Room 295G
Phone: 519-881-2111 X31135
Fax: 519-881-3420



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

Ethics Officer to Contact for Further Information

Janice Sutherland
(jsutherk@uwo.ca)

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