Rec'd Nov. 27/12

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

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

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

Donna Murrell

Jeffrey Gaudet

Francisco Martinez

Patrick Lim

Yonathan Araya

Matthew Lowerison

Amanda Hamilton

Gregory Dekaban

Mariama Henry

Yuhua Chen

Vasiliki Economopoulos

Christiane Mallet

Emeline Ribot

Additional Personnel
(Please list additional personnel and their Biosafety training dates here)

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	DH5alpha E.coli	
Approved Primary and Established Cells	Human (primary) mesenchymal stem cells. Human (established): THP-1, FaDu Luc2.11, MDA-MB-231(PA), MDA-MB-231BR, MDA-MB-435S, NK-92MI, PC-3, LNCaP clone FGC, A2058, KHYG-1, C4-2B. Mouse	Mouse mesenchymal stem cells GFP positive
Approved Use of Human Source Material	Human blood (whole) or other body fluid	
Approved Genetic Modifications (Plasmids/Vectors)	PCDNA3.1 (+) 231PA [mms6 or magA], PEYFP-C1 [magA, YFP], pDsRed monomer-Hyg-N1 (231 Br) [mms6 or magA]	

Approved Use of Animals	Nu/Nu (nude), SCID and C57B1/6 mice	
Approved Biological Toxin(s)	Pertussis	
Approved Gene Therapy		
Approved Plants and Insects		

As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder: Paula Foster

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

Date of Last Biohazardous Agents Registry Form: Feb 17, 2012

Date of Last Modification (if applicable): 11/22/2012

BioSafety Officer(s)*: Ronald Wasserfall Dec. 11/12

***For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.**

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

To Whom it May Concern,

I am writing to modify the biosafety protocol for Dr. Paula Foster's lab. The proposed work involves the mouse mesenchymal stem GFP positive cell line. The cells will be cultured and labeled with contrast agents to permit their detection by magnetic resonance imaging. If the cell labeling is effective then the cells will be it is injected/implanted into mice for a stem cells research.
The information of the cell line is attached.

Please let me know if you have questions or comments.

Regards,

Yuhua chen.

Mailing Address:
Dr. Paula Foster
Attn: Yuhua Chen/Cat Ramsay
P.O. Box 5015
100 Perth Dr
London ,ON
N6A 5K8
Phon# 519-663-5777 Ext: 24133
Fax# 519-931-5224
Email: Ychen@robarts.ca

cyagen
We help you discover life

www.cyagen.com
Tel: 800-921-8930; Fax: 408-400-0565
574 East Weddell Drive, Suite 6, Sunnyvale, CA 94089

December 4th, 2012

Ron Noseworthy, MCIC CRSP
Robarts Research Institute
Schulich School of Medicine and Dentistry
Western University
100 Perth Drive
London, ON N6A 5K8

Dear Mr. Noseworthy,

This is Isabel Mo from the Cell Biology Department of Cyagen Biosciences. With regards to your questions about our product OriCell™ C57BL/6 Mouse Mesenchymal Stem Cells with GFP (Catalog No. MUXMX-01101), please find the answers below:

OriCell™ C57BL/6 Mouse Mesenchymal Stem Cells with GFP (Catalog No. MUXMX-01101) should be handled under at least **Biosafety Level 1** (BSL-1) containment.

We use the third-generation "self-inactivating" virus system, which includes one target vector and three helper plasmids. This system is optimized on the basis of the second-generation system (three plasmids) and is much safer. Please note that in this system, the accessory protein gene *tat* is deleted, and the envelope gene is replaced with a *VSV-G* and is encoded on a third plasmid.

By splitting the system into 4 plasmids (3 helper plasmids and 1 targeting vector), the number of recombination events required to form a complete replication-competent virus increases, thus reducing the biohazard risk. To date, there are no known cases where this type of system has produced replication-competent lentivirus. (Katia Pauwels, 2009)

If you have any questions, please feel free to contact us.

Sincerely,

Isabel Mo
Cell Biology Department Manager
Cyagen Biosciences

Please find the MSDS for the C57BL/6 Mouse Mesenchymal Stem Cells with GFP (MUBMX-01101). Regarding the vector used to transduce these MSCs: the lentivirus vector with the EF1a promoter is used to express GFP, constitutively expressed in cytoplasm.

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Material Safety Data Sheet

Revision Date: 03-Feb-2012

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code MUBMX-01101
Product name C57BL/6 Mouse Mesenchymal Stem Cells with GFP

Company/Undertaking Identification

Cyagen Biosciences Inc.
574 East Weddell Dr, Suite 6
Sunnyvale, CA 94089, U.S.A.
Tel: 800-921-8931

For research use only

2. COMPOSITION/IN FORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	10

The product contains no substances which at their given concentration, are considered to be hazardous to health.

3. HAZARDS IDENTIFICATION

Emergency Overview
Components of the product may be absorbed into the body through the skin

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ **Potential Health effects**

Eyes	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Impervious butyl rubber gloves. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes. Nitrile gloves are not recomended. Some brands of Nitrile gloves have breakthrough times of five minutes.
Eye protection	Safety glasses with side-shields
Skin and body protection	Light weight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal, rat/rab bit)	LD50 (inhalation, rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eye	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available

Sensitization	No information available
Target Orcia Effects	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Reglulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CIFIR 61)
This product does not contains HAPs.

U.S. State Reaulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
D2B Toxic materials

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Cyagen Biosciences Inc. cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

cyagen *We help you discover life*

User Manual

OriCell™ Strain C57BL/6 Mouse Mesenchymal Stem Cells with GFP(MSCs/GFP)

Cat. No. MUBMX-01101

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Table of Contents

CONTENTS AND STORAGE

Product Name	Strain C57BL/6 Mouse Mesenchymal Stem Cells with GFP
Catalog No.	MUBMX-01101
Amount per Vial	1×10^6 Cells
Cryopreserved At	Ninth Passage
Storage Condition	Liquid Nitrogen

CAUTION: **Please handle this product as a potentially biohazardous material. This product contains dimethyl sulfoxide (DMSO), a hazardous material, in the freezing medium.**

PRODUCT INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into a variety of cell types including osteocytes, adipocytes, and chondrocytes. MSCs proliferate quickly and are capable of generating a local immunosuppressive microenvironment, thus contributing to their wide application potentials in tissue engineering, cell therapy, and gene therapy.

OriCell™ C57BL/6 Mesenchymal Stem Cells with GFP are derived from the bone marrow of C57BL/6 mice, cultured as monolayer, and then have been transfected with a lentiviral construct containing a GFP expression motif. They have a strong capacity for self-renewal while maintaining their multipotency.

In addition, these cells have been tested for:

- Exogenous Factors: bacterial/fungal contamination, mycoplasma contamination, and endotoxin contamination.
- Characteristics: post-thaw viability, cell cycle, verification of undifferentiated state, and differentiation potential.

This product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

CELL CHARACTERISTICS AND IDENTITY

- Strong capacity to expand. Can be passaged at least 5 times.

- Multipotent differentiation ability along the osteogenic, chondrogenic, and adipogenic lineages.

PRODUCT APPLICATIONS

Strain C57BL/6 Mouse MSCs/GFP have become a popular research target due to their potential use in regenerative medicine and tissue engineering (in areas such as cardiovascular, neural, and orthopedic disease).

OriCell™ Strain C57BL/6 Mouse MSCs/GFP can be used as cell models to evaluate the immunoreactions, proliferation, immigration, and differentiation of MSCs both *in vivo* and *in vitro*.

GENERAL HANDLING PRINCIPLES

1. Aseptic handling of the product is necessary throughout.
2. Once the cells have been established, always freeze several vials of OriCell™ Strain C57BL/6 Mouse MSCs/GFP as a backup.

Note: **The OriCell™ Strain C57BL/6 Mouse MSCs/GFP can be frozen/thawed at least two times.**

3. For all studies, it is strongly recommended to use cells that are at, or under, an original passage number of 10.
4. For general maintenance of cells, we recommend the seeding density to be $2.0\text{-}3.0\times10^4$cells/cm^2.
5. For general maintenance of cells, we recommend that the medium is changed if it becomes acidic (the pH indicator in the medium appears yellow). In general, change the growth medium every three days.
6. Do not let OriCell™ C57BL/6 MSCs/GFP overgrow as it will result in contact inhibition. When the cells are 80-90% confluent, subculturing the cells is strongly recommended.

Note: **We strongly recommend the use of OriCell™ culture media and other related reagents for optimal results.**

THAWING AND ESTABLISHING OriCell™ Strain C57BL/6 Mouse MSCs/GFP

Materials Required

- OriCell™ Mouse Mesenchymal Stem Cell Growth Medium (Cat. No.MUXMX-90011)

Thawing and Establishing Strain C57BL/6 Mouse MSCs/GFP

1. Pre-warm the fully supplemented (complete) OriCell™ Mouse MSC Growth Medium to 37°C.
2. Add 9 mL of OriCell™ Mouse MSC Growth Medium to a 15 mL conical tube.
3. Remove the cryovial of OriCell™ Strain C57BL/6 Mouse MSCs/GFP from liquid nitrogen. Quickly thaw the vial in a 37°C water bath until the last ice crystal disappears. For optimal results, be sure to finish the thawing procedure within 3 minutes. Be careful not to submerge the entire vial. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

Note: **Results will be less than optimal if the cells are thawed for more than 3 minutes.**

4. As soon as the cells are completely thawed, disinfect the outside of the cryovial with 70% v/v ethanol.
5. Use a pipette to transfer the cells to the 15 mL conical tube containing OriCell™ Mouse MSC Growth Medium inside a biosafety cabinet. Be careful not to introduce any bubbles during the transfer process.
6. Rinse the vial with 1 mL of the medium to reduce cell loss. Subsequently transfer this 1 mL of cell suspension into the conical tube.
7. Gently mix the cell suspension by slowly pipetting up and down. Be careful not to introduce any bubbles.
8. Centrifuge the cell suspension at 250 x *g* for 5 minutes.
9. Carefully aspirate off as much of the supernatant as possible and add 2-3 mL of fresh OriCell™ Mouse MSC Growth Medium (pre-warmed to 37°C).
10. Gently resuspend the cells in OriCell™ Mouse MSC Growth Medium.
11. Seed the cells into a T25 flask and add a sufficient amount of OriCell™ Mouse MSC Growth Medium. Gently rock the culture flask to evenly distribute the cells.
12. Incubate the flask at 37°C inside a 5% CO_2 humidified incubator.
13. The next day, change the medium with fresh growth medium (pre-warmed to 37°C).
14. Change the growth medium every three days thereafter.
15. When the cells are approximately 80-90% confluent, they can be dissociated with Trypsin-EDTA and passaged.

Note: Changing Medium

1. **Warm an appropriate amount of medium to 37°C in a sterile container. Replace the spent medium with the pre-warmed, fresh medium. Once completed, return the flask to the incubator.**
2. **Avoid repeated warming and cooling of the medium. If the entire content is not needed for a single procedure, transfer only the required volume to a sterile secondary container.**

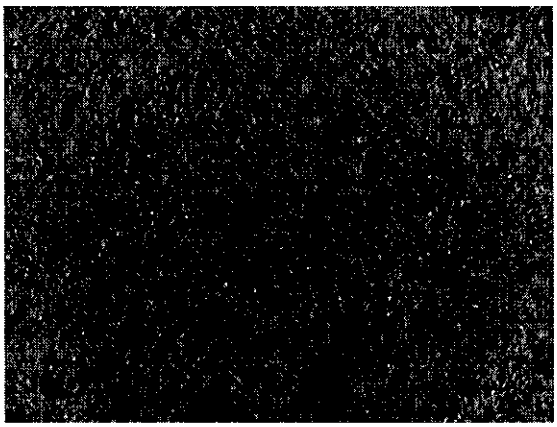

Fig. 1. OriCell™ Strain C57BL/6 Mouse Mesenchymal Stem Cells with GFP are established.

PASSAGING OriCell™ Strain C57BL/6 Mouse MSCs/GFP

Materials Required

- Trypsin-EDTA (Cat. No. TEDTA-10001)
- Phosphate-Buffered Saline (1×PBS) (Cat. No. PBS-10001)
- OriCellTM Strain C57BL/6 Mouse Mesenchymal Stem Cells with GFP (Cat. No. MUBMX-01101)
- OriCell™ Mouse Mesenchymal Stem Cell Growth Medium (Cat. No. MUXMX-90011)

Passaging OriCell™ Strain C57BL/6 Mouse MSCs/GFP

1. Pre-warm the OriCell™ Mouse MSC Growth Medium, 1×PBS, and Trypsin-EDTA solution to 37°C.
2. Carefully aspirate the spent medium from the 80-90% confluent monolayer of MSCs.
3. Add 1×PBS (6 mL for T75 flask, 3 mL for T25 flask). Be careful not to disturb the monolayer. Gently rock the flask back and forth to rinse the monolayer.
4. Aspirate 1×PBS off and discard.
5. Repeat steps 3-4 two or three times.
6. Add Trypsin-EDTA solution (1.5 mL for T75 flask, 0.5 mL for T25 flask). Gently rock the flask back and forth to ensure that the entire monolayer is covered with the Trypsin-EDTA solution. Allow trypsinization to continue until the majority of the cells (approximately 80%) are rounded up. At this point, gently tap the side of the flask to release the majority of cells from the culture flask surface.

Important: **Avoid leaving cells exposed to the trypsin longer than necessary. Care should also be taken that the cells are not forced to detach prematurely as this may result in clumping.**

7. After the cells are visibly detached, immediately add the pre-warmed OriCell™ Mouse MSC Growth Medium (6 mL for T75 flask, 3 mL for T25 flask) to neutralize the trypsinization.
8. Gently pipette the medium over the cells to dislodge and resuspend the cells. Repeat 5-6 times until all the cells are dissociated from the flask and evenly dispersed into a single cell suspension.
9. Transfer the dissociated cells into a 15 mL conical tube.
10. Centrifuge at 250 x *g* for 5 minutes.
11. Carefully aspirate off as much of the supernatant as possible.
12. Add 2 mL of OriCell™ Mouse MSC Growth Medium to the conical tube and gently resuspend the cells thoroughly.
13. Plate the cells into appropriate flasks. OriCell™ Strain C57BL/6 Mouse MSCs/GFP can be split at 1:2 or other appropriate ratios.
14. Add an appropriate amount of medium to the cells. Incubate the cells at 37°C inside a 5% CO_2 humidified incubator.

Note: **Care should be taken to avoid introducing bubbles during pipetting.**

Additional Tips

Time to Change Medium

It is recommended to change the culture medium whenever the medium becomes acidic, even if the cells do not reach 80-90% confluency. The pH indicator in the culture medium will appear yellow when acidic. In general, change the growth medium every three days.

Time to Subculture

When OriCell™ Strain C57BL/6 Mouse MSCs/GFP are 80-90% confluent, it is recommended that the cells be subcultured. Do not let the cells overgrow as it will result in contact inhibition.

OriCell™ Strain C57BL/6 Mouse MSCs DIFFERENTIATION USING OriCell™ DIFFERENTIATION MEDIA

OriCell™ Strain C57BL/6 Mouse MSCs/GFP can differentiate into a variety of cell types including osteocytes, adipocytes, and chondrocytes.

Osteogenic Differentiation

Materials Required

OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium (Cat. No. GUXMX-90021)

Osteogenesis Protocol

***Note*: The protocol listed below is for 6-well tissue culture plates.**

1. Culture the OriCell™ Strain C57BL/6 Mouse MSCs/GFP in OriCell™ Mesenchymal Stem Cell Growth Medium at 37°C in a 5% CO_2 humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with Trypsin-EDTA (Cat. No. TEDTA-10001).
3. Reseed the MSCs in the growth medium at 3×10^3cells/cm^2 in a 6-well tissue culture plate pre-coated with 0.1% gelatin solution.
4. Incubate the cells at 37°C inside a 5% CO_2 humidified incubator.
5. After 24 hours, when cells are approximately 60-70% confluent, carefully aspirate off the growth medium from each well and add 2 mL of OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium.
6. Feed cells every 3 days for 2-4 weeks by completely replacing the medium with fresh OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium.
7. After 2-4 weeks of differentiation, cells can be fixed and stained with alizarin red S.

Alizarin Red S Staining Analysis

1. After the cells have differentiated, remove the osteogenic differentiation medium from the wells and rinse with 1x phosphate-buffered saline (PBS). Fix cells with 2 mL of 4% formaldehyde solution for 30 minutes.
2. Rinse wells twice with 1x PBS. Stain the cells with 1 mL alizarin red S working solution for 3-5 minutes.
3. Rinse wells 2-3 times with 1x PBS.
4. Cells can now be visualized and analyzed under a microscope.

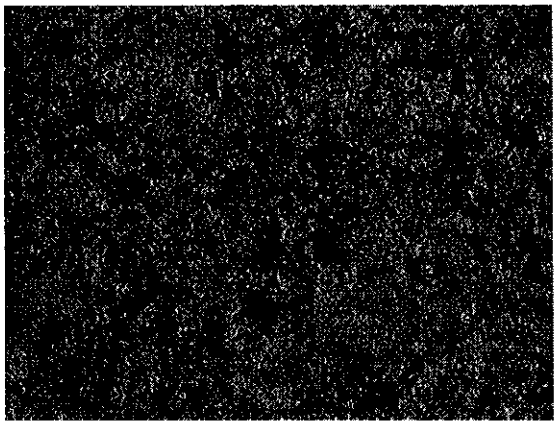

Fig. 3 OriCell™ Strain C57BL/6 Mouse MSCs/GFP are differentiated into osteocytes and are stained with alizarin red S.

Adipogenic Differentiation

Materials Required

OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation Medium (Cat. No. GUXMX-90031)

Adipogenesis Protocol

***Note*: The protocol listed below is for 6-well tissue culture plates.**

1. Culture the OriCell™ Strain C57BL/6 Mouse MSCs/GFP in the OriCell™ Mesenchymal Stem Cell Growth Medium at 37°C in a 5% CO_2 humidified incubator.
2. When cells are approximately 80-90% confluent, they can be dissociated with Trypsin-EDTA (Cat. No. TEDTA-1000).
3. Reseed the MSCs in growth medium at 2x104 cells/cm2 in a 6-well tissue culture plate with a medium volume of 2 mL per well.
4. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
5. Feed the cells every 3 days until they are 100% confluent or post-confluent. Induction of adipogenic differentiation 3-5 days post-confluency is strongly recommended.
6. When the cells are 100% confluent or post-confluent, carefully aspirate off the spent growth medium from the wells and add 2 mL of MSC induction medium per well.
7. Three days later, change the medium to MSC maintenance medium by completely replacing the spent induction medium.
8. 24 hours later, change the medium back to MSC induction medium.
9. To optimally differentiate MSCs into adipogenic cells, repeat the cycle of induction and maintenance at least three times.
10. After three to five cycles of induction and maintenance, culture the cells in maintenance medium for an additional 7 days. During this 7-day period, change the medium every 3 days.

Oil Red O Stain Analysis

1. After the cells have differentiated, remove the MSC maintenance medium from the wells and rinse with 1x phosphate-buffered saline (PBS). Fix cells with 2 mL of 4% formaldehyde solution for 30 minutes.
2. Rinse wells twice with 1x PBS and stain cells with 1 mL of oil red O working solution (3:2 dilution with distilled water and filter with filter paper) for 30 minutes.
3. Rinse wells 2-3 times with 1x PBS.
4. Cells can now be visualized and analyzed under a microscope.

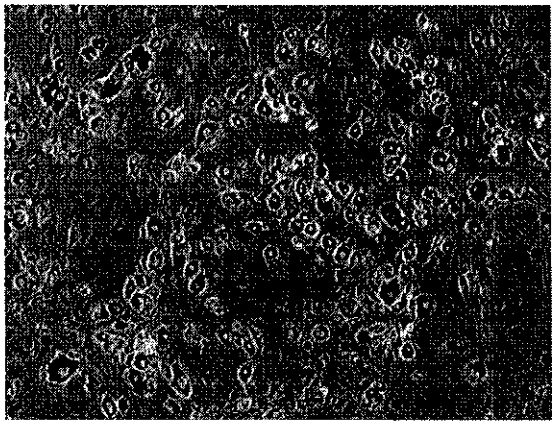

Fig.4 OriCell™ Strain C57BL/6 Mouse MSCs/GFP are differentiated into adipocytes and are stained with oil red O.

Chondrogenic Differentiation

Materials Required

OriCell™ Mesenchymal Stem Cell Chondrogenic Differentiation Medium

(Cat. No. GUXMX-90041)

Chondrogenesis Protocol

1. Calculate the total number of MSC pellet cultures required for your experiment (2.5×10^5 MSCs are needed to form each chondrogenic pellet). Transfer this amount of cells into an appropriate culture tube.
2. Wash the MSCs with Incomplete Chondrogenic Medium. Centrifuge the cells at 150 x g for 5 minutes at room temperature and then aspirate off the supernatant. Resuspend the cells in 1 mL of Incomplete Chondrogenic Medium per 7.5×105 cells. Centrifuge again at 150 x g for 5 minutes and then aspirate off the medium.
3. Resuspend the MSCs in Complete Chondrogenic medium to a concentration of 5.0×105 cells/mL.
4. Aliquot 0.5 mL (2.5×105 cells) of the cell suspension into 15 mL polypropylene culture tubes. Centrifuge the cells at 150 x g for 5 minutes at room temperature. DO NOT aspirate the supernatant or resuspend the pellet.
5. Loosen the caps of the tubes one half turn in order to allow gas exchange, and incubate the tubes at 37°C in a humidified atmosphere of 5% CO2. Do not disturb the pellets for 24 hours.
6. Feed the cell pellets every 2-3 days by completely replacing the medium in each tube (to avoid aspirating the pellets when aspirating the medium, attach a sterile 1-200µL pipette tip to the end of the aspirating pipette). Add 0.5 mL of freshly prepared Complete Chondrogenic Medium to each tube.
7. After replacing the medium, flick the bottom of the tube to ensure that the pellet is free floating. Loosen the caps and return the tubes to the 37°C incubator.
8. Chondrogenic pellets should be harvested after 14-28 days in culture. Pellets may be formalin-fixed and paraffin-embedded for alcian blue stain analysis.

Alcian Blue Staining Procedure

1. The tissue sample should be formalin-fixed and paraffin-embedded already.
2. Staining procedure:
   a) Deparaffinize slides and hydrate to distilled water.
   b) Stain in alcian blue solution for 30 minutes.
   c) Wash in running tap water for 2 minutes.
   d) Rinse in distilled water.
   e) Visualize under a light microscope and capture images for analysis. Blue staining indicates synthesis of proteoglycans by chondrocytes.

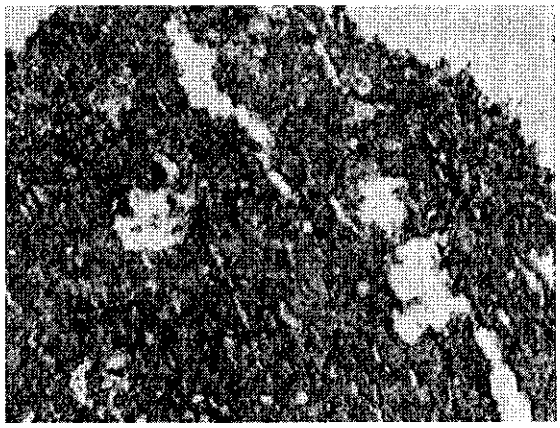

Fig.5 OriCell™ Strain C57BL/6 Mouse MSCs/GFP are differentiated into cartilages and are stained with alcian blue.

CRYOPRESERVATION OF CELLS USING OriCell™ CRYOPRESERVATION MEDIA

OriCell™ NCR Protein-Free Cryopreservation Medium (Cat. No. NCPF-10001) is a protein-free, ready-to-use freezing medium. Its chemically-defined and protein-free formulation has been optimized to stem cells and primary cells, thus greatly enhancing the viability and integrity of these cells by protecting them from damage during the one-step freeze-thaw procedure. Unlike other conventional freezing media, which require a slow programmed freeze, this product allows the cells to be directly frozen at -80°C.

Cryopreservation

Note: **Change the culture medium with fresh growth medium 24 hours before freezing.**

1. Collect cells that are in the logarithmic growth phase. Perform a cell count to determine the viable cell density.
2. Centrifuge the cells for 3-5 minutes at 250 x g and 20°C. Remove and discard the supernatant using a pipette.
3. Resuspend the cell pellet in the OriCellTM NCR Protein-Free Cryopreservation Medium at a cell density of 105-106 cells/mL.
4. Dispense aliquots of the cell suspension into cryogenic storage vials that are properly labeled.
5. Place the vials directly in a -80°C freezer. After 24 hours, transfer the frozen vials to liquid nitrogen for long-term preservation

APPENDIX

Troubleshooting

The table below lists some potential problems and solutions for culturing MSCs.

	The storage condition does not meet the requirements	Purchase a replacement and store in liquid nitrogen for long-term preservation.
	Thawing of the cells takes too long	Thaw cells for no more than 3 minutes.
	Cells are incompletely recovered after thawing	After aspirating off medium, wash the tube with culture medium twice and transfer all of the cells to the dish.
	Cells are handled roughly	Care should be taken to avoid introducing bubbles during pipetting. Also avoid vortexing and high-speed centrifugation
	Medium is not pre-warmed	Warm medium to 37°C before recovery.
	Mycoplasma contamination	Discard the cells in question and disinfect the laboratory environment before recovering the next batch of cells.
	Over digestion	Wash the cells with PBS 2-3 times to remove serum prior to trypsinization (serum will inhibit the function of trypsin).
		Control the digestion time.
	Plating density is too low	Increase the plating density.
	Inappropriate serum and medium	Use Cyagen tailor-made culture media. If other serum and media products are used, please perform validation to ensure compatibility.
	Dead cells are not removed promptly	Change the medium next day after recovery to ensure removal of all dead cells.
	Cell Contamination	Discard the cells in question and disinfect the laboratory environment before recovering the next batch of cells.
	Plating density is too low	Some stem cells can secrete factors to support cell growth. Therefore, a certain degree of plating density must be maintained; otherwise, it will lead to cell proliferation slow down and cell aging.

RELATED PRODUCTS

Product	Catalog Number
OriCell™ Mouse Mesenchymal Stem Cell Growth Medium	MUXMX-90011
OriCell™ Mesenchymal Stem Cell Osteogenic Differentiation Medium	GUXMX-90021
OriCell™ Mesenchymal Stem Cell Adipogenic Differentiation Medium	GUXMX-90031
OriCell™ Mesenchymal Stem Cell Chondrogenic Differentiation Medium	GUXMX-90041
Trypsin-EDTA	TEDTA-10001
Phosphate-Buffered Saline (1xPBS)	PBS-10001
OriCell™ NCR Protein-Free Cryopreservation Medium	NCPF-10001

REFERENCES

Alexandra Peister, Jason A. Mellad, and Benjamin L, Larson. (2004) Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *BLOOD* 1: 1662-1668.

Masoud Soleimani, and Samad Nadn. (2009) A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. *NETURE* 4: 102-106.

Philippe Tropel, Daniele Noel, and Nadine Platet. (2004) Isolation and characterisation of mesenchymal stem cells from adult mouse bone marrow. *Experimental Cell Research* 295: 395-406.

Lindolfo da Silva Meirelles, Pedro Cesar Chagastelles, and Nance Beyer Nardi. (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *Journal of Cell Science* 119: 2204-2213.

TECHNICAL SUPPORT

Please visit the Cyagen website at www.cyagen.com for technical resources, additional product information, and special offers. You may also write, email, call, or fax to us at:

Cyagen Biosciences, Inc.

574 East Weddell Drive, Suite 6

Sunnyvale, CA 94089, U.S.A.

Email: service@cyagen.com

Tel: 800-921-8930

Fax: 408-400-0565

Material Safety Data Sheets (MSDSs) are available upon request.

The Certificate of Analysis (CoA), which provides detailed quality control information for each product, is also available at the Cyagen website.

The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

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

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Paula Foster**
DEPARTMENT:	**Imaging/Medical Physics**
ADDRESS:	**Robarts Research Institute 100 perth dr Rom2276**
PHONE NUMBER:	**24040**
EMERGENCY PHONE NUMBER(S):	**24040**
EMAIL:	**pfoster@imaging.robarts.ca**

Location of experimental work to be carried out :

Building :	**RRI**	Room(s):	**2276,2222,2245B,2245E**
Building :	**UH**	Room(s):	**L1H1.5**
Building :		Room(s):	

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR, NCIC, OCIR, MSSOC, ORF**
GRANT TITLE(S): **See attached documents**
UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Yuhua Chen	**ychen@robarts.ca**	**attached**
Catherin McFadden	**mcfadden@imaging.robarts.ca**	**attached**
Amanda Hemilton	**hamilton@imaging.robarts.ca**	**attached**
Emeline Ribot	**eribot@imaging.robarts.ca**	**attached**
Gabrielle Siegers	**gsiegers@imaging.robarts.ca**	**attached**
Laura Gonzalez Lara	**gonzalez@imaging.robarts.ca**	**attached**

Christiane Mallett	cmall@imaging.robarts.ca	attached
Vasiliki Economopoulos	vecon@imaging.robarts.ca	attached
Mariama Henry	mhenry@imaging.robarts.ca	attached
Matthew Lowerison	mloweri@imaging.robarts.ca	attached
Gregory A. Dekaban	dekaban@robarts.ca	attached
Yonathan Araya	yaraya@uwo.ca	attached
Patrick Lim	hlim62@uwo.ca	attached
Francisco (Paco) Martinez	fmartine@imaging.robarts.ca	attached

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

All cell lines will be treated in the same fashion.
Cells will be subcultured aseptically in a biological cabinet. They will be labeled with super paramagnetic iron oxide particles , if labeling is successful, then the cells will be injected into the animals. The remaining cells will be disposed of bleach. The methods of injections include subcutneous, intranodal (lymph node), intracardiac, and intravenous. The injection will be performed in the room 2276 at RRI. The mice will then be housed at ACVS and periodically scanned with MRI at RRI 3T room or University Hospital's 1.5T MRI facility. After reaching the end points, animals will be sacrificed, the tissues of interest removed, and placed into formalin for futher ex vivo scaning or tissue processing. Remaining tissues will be disposed of at the ACVS facility for incineration.

Pertussis Toxin:
The pertussis toxin will be administered to mice to induce experimental autoimmune encephalomyelitis(EAE). After an intraperitoneal injection(ip), pertussis toxin will cause proliferation of Tcells , which is important in the induction process of EAE in mice. Each mouse of experiment goup will be recived 200ng pertussis toxin via ip. The injection will be done by an experienced member of the lab in the room 2276 at RRI. Animals will be housed at ACVS and imaged with MRI at RRI 3T room. Pertussis Toxin that will be kept in a locked 4-degree fridge drawer at all times unless it is in use. A small aliquot of a dilution should be made when required. A designated individual such as the laboratory supervisor should make dilutions. Also amount of pertussis toxin that is taken based on animal weight, that amount will be recorded. Approximations should be avoided, as there will be a discrepancy in the amount left in the vial and what is indicated in the records. An SOP has been created(attached).

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

The research performed in the Foster lab is focused on the use of MRI to detect and track cells in preclincial models of disease. Various strategies for labeling cells with iron oxide nanoparticles are developed in the lab. A variety of cell types have been labeled (cancer cells, immune cells, stem cells) for use in these projects. Iron labeled cells are injected or transplanted into mouse models of disease and MRI is used to monitor the cell survival, location and persistence over time (typcially weeks). One focus in the lab is tracking cancer cell metastasis. Another is tracking the fate of stem cells tranplanted in a model of multiple sclerosis. At the end of the imaging experiments the mice are euthanized and the tissues of interest are fixed and prepared for histological analysis to confirm the MRI findings.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species ______________________
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:

Full Scientific Name of Biological Agent(s)* (Be specific)	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	[illegible]
DH5α E.Coli strain	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.5L**	**Invitrogen**	[illegible]
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ______________________

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	☒ Yes ☐ No	**human blood**	Not applicable
Rodent	☐ Yes ☒ No		
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**attached**	**attached**	**attached**
Rodent	☒ Yes ☐ No	**attached**	**attached**	**attached**
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ 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 ☒ 1 ☒ 2 ☐ 2+ ☐ 3

Additional Comments: _____

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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	**Various health donors**	☐ Yes ☒ Unknown	**n/a**	☐1 ☒2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid	**n/a**	☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)	**n/a**	☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)	**n/a**	Not Applicable		Not Applicable

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
no	**see attached sheet**	**Clontech**	**magA or mms6**	**no**	**no**	**no change**

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☒ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: **Raji cells contain EBV**

6.0 Human Gene Therapy Trials

6.1 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 7.0

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Nu/Nu(nude) , SCID and C57B1/6 mice**

7.3 AUS protocol **# 2900-042, 2010-210**

7.4 List the location(s) for the animal experimentation and housing. **RRI 2276 and ACVS**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☒ YES, specify: **All**

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: **The bio-agents are injected into the animal that will induce the animal cancer model and will not be shed by the animal.**

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☒ NO

8.3 If **YES**, please specify the animal(s) used:

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

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Pertussis Toxin**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **18ug/kg. no information for human**

9.4 How much of the toxin or hormone is handled at one time*? **200ng /each mouse**

9.5 How much of the toxin or hormone is stored*? **50-70ug**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **Pertussis toxin**

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☒ YES, country of origin **Germany** ☐ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☒ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☒ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☒ YES, please provide permit # **P-11-0060** ☐ NO

13.0 Training Requirements for Personnel Named on Form

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

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Paula Foster** ***Date:*** **Nov 14.2011**

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **Rm#2222, Rm#2276**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **Bio-RRI-0032**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
The injection will be done by a experienced member of Lab. An SOP has been created.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Paula Foster** ***Date:*** **Nov 14 2011**

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 17 Feb 12

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Feb 16, 2012

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Ronald Newcombe
Date: Jan. 26, 2012

Approval Number: BIO-RRI-0032 Expiry Date (3 years from Approval): Feb 16, 2015

Special Conditions of Approval:

Grant project titles

Early and Targeted Detection of Metastatic Cancer in the Lymphatic System Using Cellular Magnetic Resonance Imaging and Nanotechnology(NCIC)

One Millimetre Cancer Challenge (1mmCC), "Tracking Cancer(OICR) Vaccines"

The Use of Cellular MRI to Evaluate Stem Cell Transplantation in a Model of Multiple Sclerosis(MSSOC)

Ontario pre-clinical imaging consortium ----Foster/Rutt/McKenzie (ORF)

Foster Lab Training Cetrificate Inventory

	BioSafety Training	WHMIS Training	Waste Mamagement Safety Training	Radiation Safety Training
Amanda Hamilton	10-Feb-11	19-Jan-11	26-Oct-11	
Catherine Ramsay	20-May-11	19-Mar-09	20-May-09	
Christiane Mallett	13-Jun-06	01-Apr-11		
Emeline Ribot	07-May-09	03/04/09	05-Jul-09	
Gabrielle Siegers	24-Sep-10	04-Oct-11	27-Sep-10	16-Sep-10
Jonatan Snir		05-May-11	04-May-11	
Laura Gonzalez Lara	07-Jan-11	07-Jan-10		
Mariama Henry	26-Oct-11	26-Sep-11		
Vasiliki Economopoulos	04-Oct-08	22-Sep-11	26-Sep-11	
Yuhua Chen	21-Nov-07	29-Mar-03	08-Mar-11	
Gregory A. Dekaban	02/06/05	16/10/2007		
Metthew Lowerison	26-Aug-11	03-Aug-11	22-Sep-11	
Yonathan Araya	24-Nov-11	27-Jan-11	16-Nov-11	
Patrick Lim	23-Nov-11		23-Nov-11	
Francisco M. Martinez		01-Jun-11		

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

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

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

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

Canada

Info on Cell Line(s)

Cell line will be numbered. These numbers will be indicated on the form due to limited space on the form.

Name Type	Supplier	Biosafety Level
(1) THP-1 (human)	ATCC(TIB-202)	1
(2) B16F10 (Rodent)	Ann Chambers LRCC (collaboration)	1
(3) Glioma-261 (Rodent)	National Cancer institute (NCI)	1
(4) Glioma-261RFP (Rodent)	NCI	1
(5) FaDu Luc2.11 (Human)	Becton Dickinson Technologies (Collaboration)(Purchase from Attc(HTB-43) before modification done by BD)	1
(6) MDA-MB-231-231PA(human)	Ann Chambers LRCC(collaboration) (HTB-26)	1 1
(7) MDA-MB-231BR(human)	Ann Chambers LRCC (HTB-26)	1
(8) MDA-MB-231-luc-D3H2LN)	Ann Chambers LRCC(HTB-26)	1
(9) MDA-MB-435	Ann Chambers LRCC NCIDTP60	1
(10) MDA-MB-231BR eGFP	Dr. Brian Rutt's lab (collaboration) (HTB-26)	1
(11) MDA-MB-231 with mms6 pcDNA	Dr. Brian Rutt's lab (HTB-26)	1
(12) MDA-MB-231 with magA pcDNA	Dr. Brian Rutt's lab (HTB-26)	1
(13) MDA-MB-231 with pcDNA	Dr. Brian Rutt's lab (HTB-26)	1
(14) MDA-MB-231BR eGFP with mms6 pDsRed	Dr. Brian Rutt's lab (HTB-26)	1
(15) MDA-MB-231BR eGFP with magA pDsRed	Dr. Brian Rutt's lab(HTB-26)	1
(16) MDA-MB-231BR eGFP with mms6-DsRed pDsRed	Dr. Brian Rutt's lab(HTB-26)	1
(17) MDA-MB-231BR eGFP with magA-mms6 pDsRed	Dr. Brian Rutt's lab(HTB-26)	1
(18) MDA-MB-231BR eGFP with magA-DsRed pDsRed	Dr. Brian Rutt's lab(HTB-26)	1
(19) MDA-MB-231BR eGFP with pDsRed	Dr. Brian Rutt's lab(HTB-26)	1
(20)KHYG-1(human)	U of T Dr. Keating lab (collaboration (JCRB0156)	1
(21) C4-2B (human)	ViroMed Laboratories	1
(22) PC-3 (human)	NCI (CRL-1435)	
1		
(23) Melanoma A2058(human)	Cedarlane (Attc: CRL-11147)	1

All the cell lines of the above are biosafety level I.

(1) Human mesenchymal stem cells	U of T Dr. Keating lab (information is attached)	2

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Cell Biology

ATCC® Number:	TIB-202™ Order this Item	**Price:**	$279.00
Designations:	THP-1		Related Links
Depositors:	S Tsuchiya		
Biosafety Level:	1		
Shipped:	frozen		NCBI Entrez Search
Medium & Serum:	See Propagation		Cell Micrograph
Growth Properties:	suspension		
Organism:	*Homo sapiens*		Make a Deposit
Morphology:	monocyte		Frequently Asked Questions
			Material Transfer Agreement
			Technical Support
			Related Cell Culture Products
Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;		
Cellular Products:	lysozyme		Product Information Sheet
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		
Receptors:	complement (C3), expressed [58053] Fc, expressed		
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16		
Age:	1 year infant		
Gender:	male		

Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2-4 X 10(5) viable cells/ml. Subculture when cell concentration reaches 8X105) cells/ml.Do not allow the cell concentration to exceed 1 X 10(6) cells/ml. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	approximately 26 hrs
Related Products:	purified RNA:ATCC TIB-202R purified DNA:ATCC TIB-202D Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2001 Recommended serum: ATCC 30-2020 Cell culture tested DMSO: ATCC 4-X
References:	22193: Tsuchiya S, et al. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. 42: 1530-1536, 1982. PubMed: 6949641 22285: Skubitz KM, et al. Human granulocyte surface molecules identified by murine monoclonal antibodies. J. Immunol. 131: 1882-1888, 1983. PubMed: 6619543 32286: Cuthbert JA, Lipsky PE. Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. Cancer Res. 57: 3498-3504, 1997. PubMed: 9270019 32351: Huang S, et al. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. 70: 4502-4508, 1996. PubMed: 8676475 32395: Clark RA, et al. Tenascin supports lymphocyte rolling. J. Cell Biol. 137: 755-765, 1997. PubMed: 9151679 32466: Hambleton J, et al. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad. Sci. USA 93: 2774-2778, 1996. PubMed: 8610116 33031: Hsu HY, et al. Inhibition of macrophage scavenger receptor activity by tumor necrosis factor-alpha is transcriptionally and post-transcriptionally regulated. J. Biol. Chem. 271: 7767-7773, 1996. PubMed: 8631819 33088: Lucas M, Mazzone T. Cell surface proteoglycans modulate net synthesis and secretion of macrophage apolipoprotein E. J. Biol. Chem. 271: 13454-13460, 1996. PubMed: 8662812 33134: Sando GN, et al. Induction of ceramide glucosyltransferase activity in cultured human keratinocytes. J. Biol. Chem. 271: 22044-22051, 1996. PubMed: 8703011 33141: Ollivier V, et al. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. J. Biol. Chem. 271: 20826-20835, 1996. PubMed: 8702838 58053: Tsuchiya S, et al. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer 26: 171-176, 1980. PubMed: 6970727

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Cell Biology

ATCC® Number: CCL-107™ Order this Item

Price: $279.00

Designations: C6

Depositors: G Sato

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Rattus norvegicus* deposited as *Rattus* sp.

Morphology: fibroblast

Source:
Organ: brain
Disease: glioma
Cell Type: glial cell;

Cellular Products: S-100 protein; produce glyceryl phosphate dehydrogenase in response to glucocorticoids; somatotrophin

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

Receptors: glucocorticoid

Virus Resistance: poliovirus 3

Cytogenetic Analysis: Stemline number is diploid. Karyotype is stable within the stemline number and is that of a normal male.
Three cells with breaks; one with a secondary constriction, one with a dicentric, one with a rearrangement and four with terminal or centromere associations.

Comments: The glial cell strain, C6, was cloned from a rat glial tumor induced by N-nitrosomethylurea by Benda et al. after a series of alternate culture and animal passages [PubMed: 4873531]. S-100 production increases ten fold as cells grow from low density to confluency.

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 2.5%; horse serum to a final concentration of 15%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Related Links

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Technical Support
Related Cell Culture Products

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Subculturing:

Protocol:

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

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020
recommended serum:ATCC 30-2040
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

1022: Benda P, et al. Differentiated rat glial cell strain in tissue culture. Science 161: 370-371, 1968. PubMed: 4873531
25965: Lightbody JJ, et al. Establishment of differentiated clonal strains of glial brain cells in culture. Fed. Proc. 27: 720, 1968.
32720: Chen Y, et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item

Price: $279.00

Designations: B16-F10

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus*

Morphology: mixture of spindle-shaped and epithelial-like cells

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

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

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%

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Product Information Sheet

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 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

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

References:

22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987
22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res. 37: 3945-3956, 1977. PubMed: 908034
22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895, 1977. PubMed: 887927
23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. J. Natl. Cancer Inst. 60: 1217-1222, 1978. PubMed: 418183
23362: . . Nat. New Biol. 242: 148-149, 1973.
16173787: Li M, et al. Loss of intracisternal A-type retroviral particles in BL6 melanoma cells transfected with MHC class I genes. J.Gen. Virol. 77: 2757-2765, 1996. PubMed: 8922469

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Cell Biology

ATCC® Number:	HTB-43™ Order this Item	**Price:**	**$294.00**
Designations:	FaDu	**Depositors:**	SR Rangan
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
Source:	**Organ:** pharynx **Disease:** squamous cell 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.		

Related Cell Culture Products

Applications:	transfection host(Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
Cytogenetic Analysis:	(P16) hypodiploid to hypertriploid with modal number = 64
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 2 PGM1, 2 PGM3, 1
Age:	56 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The FaDu line was established in 1968 from a punch biopsy of an hypopharyngeal tumor removed from a Hindu patient. The established line was found to contain bundles of tonofilaments in the cell cytoplasm and desmosomal regions were prominent at cell boundaries.
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: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week 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%

Product Description

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Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	**Price:**	**$244.00**
Designations:	MDA-MB-231	**Depositors:**	R Cailleau
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
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.		
	Related Cell Culture Products		
Applications:	transfection host(Technology from amaxa 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.		
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		

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Cell Biology

ATCC® Number: HTB-129™ Order this Item **Price:** $279.00

Designations: MDA-MB-435S

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens*

Morphology: spindle shaped

Source: **Organ:** previously described as: mammary gland; breast
Disease: previously described as ductal carcinoma
Derived from metastatic site: pleural effusion

Cellular Products: tubulin; actin

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.

Isolation: **Isolation date:** 1976

Tumorigenic: No

DNA Profile (STR): Amelogenin: X
CSF1PO: 11
D13S317: 12
D16S539: 13
D5S818: 12
D7S820: 8,10
THO1: 6,7
TPOX: 8,11
vWA: 16,18

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Cytogenetic Analysis: modal number = 56; range = 55 to 62
The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.

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

Age: 31 years adult

Gender: female

Ethnicity: Caucasian

Comments: This cell line was originally described as a spindle shaped variant of the parental MDA-MB-435 strain isolated in 1976 by R. Cailleau, et al. from the pleural effusion of a 31 year old female with metastatic, ductal adenocarcinoma of the breast. However, recent studies have generated questions about the origin of the parent cell line, MDA-MB-435, and by extension HTB-129. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast [PubMed ID: 10700174, PubMed ID: 15150101, PubMed ID: 15679052]. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCC has responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line [PubMed ID: 12354931 and PubMed ID: 17004106].
Derivatives of HTB-129 with identities in question:
M4A4, ATCC ® CRL-2914
M4A4 GFP, ATCC ® CRL-2915
M4A4 LM3-2 GFP, ATCC ® CRL-2916
M4A4 LM3-4 CL 16 GFP, ATCC ® CRL-2917
NM2C5, ATCC ® CRL-2918
NM2C5 GFP, ATCC ® CRL-2919

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:

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

Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing: **Protocol:** Remove medium, add fresh 0.25%trypsin - 0.53 mM EDTA, rinse and remove. Place flask at room temperature (or incubated at 37C) for approximately 10 minutes or until the cells detach. Add fresh medium, aspirate and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-129D
purified RNA:ATCC HTB-129R

References:

1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337
22429: Siciliano MJ, et al. Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker. Cancer Res. 39: 919-922, 1979. PubMed: 427779
22656: Cailleau R, et al. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro 14: 911-915, 1978. PubMed: 730202
32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194
32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: 8650224
49803: Ross DT, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nature Genetics 24: 227-235, 2000. PubMed: 10700174
89918: Ellison G, et al. Further evidence to support the melanocytic origin of MDA-MB-435. Mol. Pathol. 55: 294-299, 2002. PubMed: 12354931
90826: Sellappan s, et al. Lineage infidelity of MDA-MB-435 cells: expression of melanocyte proteins in a breast cancer cell line. Cancer Res. 64: 3479-3485, 2004. PubMed: 15150101
90828: Rae JM, et al. Common origins of MDA-MB-435 cells from various sources with those shown to have melanoma properties. Clin. Exp. Metastasis 21: 543-552, 2004. PubMed: 15679052
16173093: Rae JM, et al., MDA-MB-435 cells are derived from M14 Melanoma cells - a loss for breast cancer, but a boon for melanoma research. Breast Cancer Res. Treat. 104:13-19, 2007. PubMed: 17004106.
16173545: Chambers AF. MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 69(13): 5292-5293, 2009. PubMed: 19549886.

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Cell Biology

ATCC® Number:	**CRL-2408™** Order this Item	**Price:**	**$329.00**
Designations:	NK-92MI		
Depositors:	Conkwest Inc.		
Biosafety Level:	2		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	suspension, multicell aggregates		
Organism:	*Homo sapiens* deposited as human		
Morphology:	lymphoblast		

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Source:	**Disease:** malignant non-Hodgkin's lymphoma **Cell Type:** natural killer cell; NK cell;
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.
DNA Profile (STR):	D5S818: 12. 13 D13S317: 9, 12 D7S820: 10, 11 D16S539: 11, 12 vWA: 16, 18 THO1: 6, 9.3 TPOX: 8 CSF1PO: 11, 12 Amelogenin: X, Y
Age:	50 years
Gender:	male
Ethnicity:	Caucasian

Comments:

NK-92 is an interleukin-2 (IL-2) dependent Natural Killer Cell line derived from peripheral blood mononuclear cells from a 50 year old Caucasian male with rapidly progressive non-Hodgkin's lymphoma. NK-92MI is an interleukin-2 (IL-2) independent Natural Killer Cell line derived from the NK-92 (ATCC CRL-2407) cell line by transfection. The parental cells were transfected with human IL-2 cDNA in the retroviral MFG-hIL-2 vector by particle-mediated gene transfer. The transfection is stable, most likely due to integration of the vector into genomic DNA. The cell line is cytotoxic to a wide range of malignant cells; it kills both K562 cells and Daudi cells in chromium release assays. NK-92 and derivative cell line NK-92MI have the following characteristics: surface marker positive for CD2, CD7, CD11a, CD28, CD45, CD54 and CD56 bright; surface marker negative for CD1, CD3, CD4, CD5, CD8, CD10, CD14, CD16, CD19, CD20, CD23, CD34 and HLA-DR. The parental IL-2 dependent cell line is available as CRL-2407 (NK-92). NK-92MI was shown to contain, express, and synthesize the hIL-2. A culture submitted to the ATCC in September of 1998 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with BM Cycline. The cells were assayed for mycoplasma, by the Hoechst stain, PCR and the standard culture test, after a six-week period following treatment. All tests were negative.

Propagation:

ATCC complete growth medium: The base medium for this cell line is Alpha Minimum Essential medium without ribonucleosides and deoxyribonucleosides but with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate . To make the complete growth medium, add the following components to the base medium: 0.2 mM inositol; 0.1 mM 2-mercaptoethanol; 0.02 mM folic acid; horse serum to a final concentration of 12.5%; fetal bovine serum to a final concentration of 12.5%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

Subculturing:

Protocol: Cultures can be maintained by centrifuging cells and resuspending cell pellet in fresh medium at 2 - 3 X 105 viable cells/mL. Centrifugation and full replacement of culture medium may be performed for the first subcultures. Cultures can then be maintained by addition of fresh medium. Pipet the cells up and down on the back of the flask every 2-3 days to produce a single cell suspension. Maintain cell density between 2 X 10 5 and 1 X 10 6 viable cells/ml or use a 1:3 spilt ratio.

Preservation:

Freeze medium: FBS, 90%; DMSO, 10%
Storage temperature: liquid nitrogen vapor phase

Related Products:

recommended serum: ATCC 30-2020
recommended serum: ATCC 30-2040
parental cell line: ATCC CRL-2407 (EBV po

References:

38894: Gong JH, et al. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. Leukemia 8: 652-658, 1994. PubMed: 8152260
38969: Tam YK, et al. Characterization of genetically altered, interleukin 2-independent natural killer cell lines suitable for adoptive cellular immunotherapy. Hum. Gene Ther. 10: 1359-1373, 1999. PubMed: 10365666
40184: Tam YK, et al. Immunotherapy of malignant melanoma in a SCID mouse model using the highly cytotoxic natural killer cell line NK-92. J. Hematother. 8: 281-290, 1999. PubMed: 10417052

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Cell Biology

ATCC® Number: CRL-1435™ Order this Item

Price: $279.00

Designations: PC-3

Depositors: ME Kaighn

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent (The cells form clusters in soft agar and can be adapted to suspension growth)

Organism: *Homo sapiens*

Morphology: epithelial

Source:
Organ: prostate
Tumor Stage: grade IV
Disease: adenocarcinoma
Derived from metastatic site: bone

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

Tumorigenic: Yes

Antigen Expression: HLA A1, A9

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 11
D16S539: 11
D5S818: 13
D7S820: 8,11
THO1: 6,7
TPOX: 8,9
vWA: 17

Cytogenetic Analysis: The line is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.

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Product Information Sheet

Age:	62 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **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 1:3 to 1:6 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:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020
References:	22363: Kaighn ME, et al. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol. 17: 16-23, 1979. PubMed: 447482 22470: Chen TR. Chromosome identity of human prostate cancer cell lines, PC-3 and PPC-1. Cytogenet. Cell Genet. 62: 183-184, 1993. PubMed: 8428522 26302: Ohnuki Y, et al. Chromosomal analysis of human prostatic adenocarcinoma cell lines. Cancer Res. 40: 524-534, 1980. PubMed: 7471073 32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194 32344: Umekita Y, et al. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996. PubMed: 8876218 32460: Carter RE, et al. Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. Proc. Natl. Acad. Sci. USA 93: 749-753, 1996. PubMed: 8570628 32486: Nupponen NN, et al. Genetic alterations in prostate cancer cell lines detected by comparative genomic hybridization. Cancer Genet. Cytogenet. 101: 53-57, 1998. PubMed: 9460501 32488: Geiger T, et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. Anticancer Drug Des. 13: 35-45, 1998. PubMed: 9474241 32916: Su ZZ, et al. Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen gene PCTA-1 a member of the galectin gene family. Proc. Natl. Acad. Sci. USA 93: 7252-7257, 1996. PubMed: 8692978

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C4-2B

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Cell Biology

ATCC® Number: CRL-1740™ Order this Item

Price: $279.00

Designations: LNCaP clone FGC

Depositors: JS Horoszewicz

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent, single cells and loosely attached clusters

Organism: *Homo sapiens*

Morphology: epithelial

Source:
Organ: prostate
Disease: carcinoma
Derived from metastatic site: left supraclavicular lymph node

Cellular Products: human prostatic acid phosphatase; prostate specific antigen

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

Restrictions: Distribution of this material for commercial purposes will require execution of a Non-exclusive License Agreement. At the time of placing an order, customers must send a request to licensing@ATCC.org. Orders will be shipped when Customer Service receives confirmation from our Licensing officer.

Isolation: **Isolation date:** 1977

Applications: transfection host

Receptors: androgen receptor, positive; estrogen receptor, positive [23045]

Tumorigenic: Yes

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DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 10,11
D13S317: 10,12
D16S539: 11
D5S818: 11,12
D7S820: 9.1,10.3
THO1: 9
TPOX: 8,9
vWA: 16,18

Cytogenetic Analysis: This is a hypotetraploid human cell line. The modal chromosome number was 84, occurring in 22% of cells. However, cells with chromosome counts of 86 (20%) and 87 (18%) also occurred at high frequencies. The rate of cells with higher ploidies was 6.0%.

Age: 50 years adult

Gender: male

Ethnicity: Caucasian

Comments: LNCaP clone FGC was isolated in 1977 by J.S. Horoszewicz, et al., from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old Caucasian male (blood type B+) with confirmed diagnosis of metastatic prostate carcinoma.
These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production).
The cells do not produce a uniform monolayer, but grow in clusters which should be broken apart by repeated pipetting when subcultures are prepared.
They attach only lightly to the substrate, do not become confluent and rapidly acidify the medium.
Growth is very slow.
The cells should be allowed to incubate undisturbed for the first 48 hours after subculture.
When flask cultures are shipped, the majority of the cells become detached from the flask and float in the medium.
Upon receipt, incubate the flask (in the usual position for monolayer cultures) for 24 to 48 hours to allow the cells to re-attach.
The medium can then be removed and replaced with fresh medium.
If desired, the contents of the flask can be collected, centrifuged at 300 X g for 15 minutes, resuspended in 10 ml of medium and dispensed into a single flask.

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. 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.
   Maintain cultures at a cell concentration between 1 X 10(4) and 2 X 10(5) cells/cm2.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Twice per week

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

Doubling Time: about 34 hours

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020
derivative:ATCC CRL-10995
purified DNA:ATCC CRL-1740D

References:

21889: . Models for prostate cancer. 37New York: Liss; 1980.
22410: Gibas Z, et al. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet. Cytogenet. 11: 399-404, 1984. PubMed: 6584201
23045: Horoszewicz JS, et al. LNCaP model of human prostatic carcinoma. Cancer Res. 43: 1809-1818, 1983. PubMed: 6831420
32283: Hu SX, et al. Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression. Cancer Res. 57: 3339-3343, 1997. PubMed: 9269991
33090: Boffa LC, et al. Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence. J. Biol. Chem. 271: 13228-13233, 1996. PubMed: 8662737

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Cell Biology

ATCC® Number: CRL-11147™ Order this Item **Price:** $329.00

Designations: A2058

Depositors: W Stetler-Stevenson

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: skin
Disease: melanoma
Derived from metastatic site: lymph node

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 (technology from amaxa)

Receptors:
nerve growth factor (NGF), expressed
laminin, expressed

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 10,11
D13S317: 13,14
D16S539: 9,13
D5S818: 9,12
D7S820: 11
THO1: 7,9
TPOX: 8
vWA: 14,18

Age: 43 years adult

Gender: male

Ethnicity: Caucasian

Comments: This cell line is highly invasive and provides a source of cellular invasion associated proteins (such as the 72000 dalton type IV collagenase.
Tissue inhibitor of metalloproteinase-2 [TIMP-2], autocrine motility factor and the 67000 dalton laminin receptor.

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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 1:6 to 1:12 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
recommended serum:ATCC 30-2020

References:

22590: Fabricant RN, et al. Nerve growth factor receptors on human melanoma cells in culture. Proc. Natl. Acad. Sci. USA 74: 565-569, 1977. PubMed: 265522
23263: Sherwin SA, et al. Human melanoma cells have both nerve growth factor and nerve growth factor-specific receptors on their cell surfaces. Proc. Natl. Acad. Sci. USA 76: 1288-1292, 1979. PubMed: 375235
23269: Todaro GJ, et al. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. Proc. Natl. Acad. Sci. USA 77: 5258-5262, 1980. PubMed: 6254071
23404: Stetler-Stevenson WG, et al. The activation of human type IV collagenase proenzyme. Sequence identification of the major conversion product following organomercurial activation. J. Biol. Chem. 264: 1353-1356, 1989. PubMed: 2536363
23549: Stetler-Stevenson WG, et al. Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family. J. Biol. Chem. 264: 17374-17378, 1989. PubMed: 2793861

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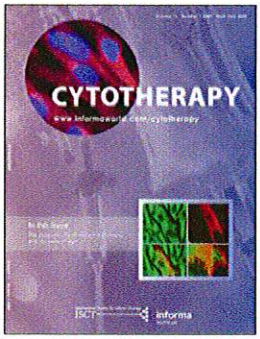

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Improved isolation protocol for equine cord blood-derived mesenchymal stromal cells

Thomas G. Koch [ab]; Preben D. Thomsen [b]; Dean H. Betts [a]
[a] Department of Biomedical Sciences, University of Guelph, Ontario, Guelph, Canada [b] Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark

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Improved isolation protocol for equine cord blood-derived mesenchymal stromal cells

Thomas G. Koch[1,2], Preben D. Thomsen[2] and Dean H. Betts[1]

[1]*Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada, and* [2]*Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark*

Background aims

A robust methodology for the isolation of cord blood-derived multipotent mesenchymal stromal cells (CB-MSCs) from fresh umbilical cord blood has not been reported in any species. The objective of this study was to improve the isolation procedure for equine CB-MSCs.

Methods

Pre-culture separation of red and white blood cells was done using either PrepaCyte®-EQ medium or Ficoll-Paque™ PREMIUM density medium. Regular FBS and MSC-qualified FBS were compared for their ability to support the establishment of putative primary MSC colonies.

Results and conclusions

Our results indicate that PrepaCyte®-EQ medium is superior to Ficoll-Paque™ PREMIUM density medium for the isolation of putative equine CB MSC and that MSC-qualified FBS may improve the isolation efficiency.

Keywords

Cord blood, horse, isolation, mesenchymal stromal cells.

Introduction

Consistent and reproducible isolation of cord blood (CB)-derived multipotent mesenchymal stromal cells (MSC) from fresh umbilical cord blood has not been reported in any study regardless of the species concerned. The highest reported isolation percentage has been 63% on selected cord blood samples. Developing a robust isolation method for these progenitor cells is crucial for the widespread acceptance of cord blood as a cell source for autologous use later in life. The objective of this study was to improve the isolation procedure for equine CB MSC by evaluating different cell separation methods and fetal bovine serum (FBS) batches. Our results indicate that PrepaCyte®-EQ medium is superior to Ficoll-Paque™ PREMIUM density medium for the isolation of putative equine CB MSC and that MSC-qualified FBS may improve the isolation efficiency.

Methods

Study design and cell line establishment

Each of five fresh CB samples was split into three groups of 42 mL, from which the nuclear or mononuclear cell (MNC) fraction was isolated by one of three methods: PrepaCyte®-EQ medium (PEQ; BioE Inc., St Paul, MN, USA), Ficoll-Paque™ PREMIUM medium (1.077 g/mL; GE Healthcare, Mississauga, ON, Canada) loaded with undiluted whole blood (FUD) and Ficoll-Paque™ PREMIUM medium loaded with diluted whole blood (FD). The manufacturer's guidelines were followed using A and B salt solutions (Figure 1). The cell suspension from each of the three separation methods was split into two groups to allow assessment of two different batches of FBS, one being regular FBS and one being 'MSC-qualified' FBS.

The CB was collected as described previously [1]. The PEQ protocol was based on the manufacturer's guidelines using 7 mL whole blood mixed with 7 mL PEQ in 15-mL tubes. In the FUD and FD groups, 7 mL sample were loaded on to 6 mL Ficoll-Paque PREMIUM density medium (1.077 g/mL) in 15-mL tubes (six tubes total) prior to cell separation, as per the manufacture's guidelines. The nucleated cells in each culture group were suspended in 10 mL isolation medium (low-glucose Dulbecco's modified Eagle medium; DMEM; Lonza, Wakersville, MD, USA), 30% regular FBS (Invitrogen, Burlington, ON, Canada) or

Correspondence to: T. G. Koch, Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1. E-mail: tkoch@uoguelph.ca

DOI: 10.1080/14653240902887259

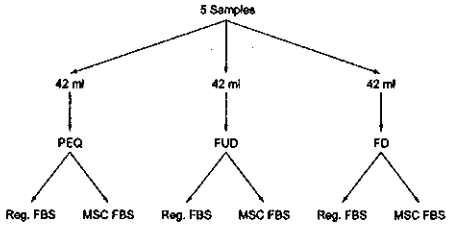

Figure 1. Study design. Each of five fresh CB samples was split into three groups of 42 mL and the nuclear or MNC fraction was isolated using the PEQ, FUD or FD protocol. Cell suspensions from each of the three separation groups were split further into two groups to allow assessment of two different batches of FBS, regular FBS (Reg. FBS) and MSC-qualified FBS (MSC FBS).

MSC-qualified FBS (Invitrogen), with low dexamethasone (10^{-7} M; Sigma, Oakville, ON, Canada), penicillin (100 IU/mL; Invitrogen), streptomycin (0.1 mg/mL; Invitrogen) and L-glutamine (2 mM; Sigma). One milliliter was submitted for automated differential cell counting. As most reported seeding densities are based on manual cell counts, which do not discriminate between granulocytes and monocytes, seeding densities were based on the total white blood cell count. Cells were seeded in six-well polystyrene plastic culture plates and incubated at 38.5°C in humidified atmosphere containing 5% CO_2 in air.

Population doubling time (PDT) was calculated from passage (P) 2 onwards as follows: PDT = (log(number of cells obtained at subculture per cm^2/[cell seeding density per cm^2)/log 2)/d], where d is the number of days in culture.

Trilineage differentiation studies

Three cryopreserved cell lines from the PEQ group were assessed for adipogenic, chondrogenic and osteogenic potency, as demonstrated previously for cells derived using Ficoll medium separation [1].

For adipogenesis, each cell line was cultured for 10 days in either continuous expansion culture medium (regular FBS), as defined above, or continuous adipogenic induction medium (BulletKit®; Lonza). The induction medium consisted of 1 µM dexamethasone, 0.5 mM 3-isobutyl-1-methyl-xanthine (IBMX), 10 µg/mL recombinant human (rh) insulin, 0.2 mM indomethacin and 10% fetal calf serum (FCS) in DMEM. The fetal calf serum included the commercial medium kit for adipogenic induction was discarded and substituted with 15% rabbit serum (Sigma). Oil Red O staining was done as described previously except rinsing and staining volumes of 200 µL were used because of the smaller well size [1]. The AdipoRed™ assay (Lonza) was used according to the manufacturer's protocol for 96-well plates with the exception of a 520-nm emission filter instead of 572 nm or 535 nm. The suitability of the 520-nm emission filter was discussed with the manufacturer's scientific product adviser prior to use. The total protein content of the AdipoRed assayed wells was determined as described previously using a DC Bio-Rad protein method (DC protein assay reagents package; Biorad, Mississauga, ON, Canada) [1].

Chondrogenic differentiation was performed using a micromass culture system [1,2]. Undifferentiated cells (2.5×10^5 cells) were cultured for 2 and 4 weeks in 0.5 mL complete chondrogenic differentiation medium (Lonza) containing 10 ng/mL transforming growth factor-beta 3 (TGF-β3). Pellets were fixed in 10% formalin, imbedded in paraffin blocks and sectioned into 5-µm sections. Hematoxylin and eosin (Sigma) as well as Safranin O (Sigma) and Alcian Blue (Sigma) staining was used to evaluate chondrogenic differentiation histologically.

Osteogenesis was induced using osteogenic induction medium (100 nm dexamethasone and 10 mM β-glycerophosphate; Sigma), 0.05 mM L-ascorbic acid-2-phosphate (Fluka Biochemika, Sigma) and 10% FBS in low-glucose DMEM (Lonza) for 10 days and compared with control cultures exposed to regular expansion medium. Osteogenic differentiation was evaluated qualitatively using Alizarin Red S staining and semi-quantitatively by alkaline phosphatase, calcium and protein assays, as reported elsewhere [1].

Statistical analysis

Data were analyzed using ANOVA, Box Cox transforms, linear modeling and contrast methods as applicable.

Results and Discussion

Significantly more MNC were isolated in the PEQ group compared with the FUD and FD groups (Figure 2A). The average total numbers of MNC seeded from each of the processed 42 mL cord blood within each group were as follows: PEQ, $4.5.0 \times 10^8$ cells (range 2.43×10^8 – 8.91×10^8); FUD, 2.88×10^7 cells (range 9.0×10^6 – 5.4×10^7); FD, 2.52×10^7 cells (range 9.0×10^6 – 5.4×10). Significantly more colonies were established in the PEQ group

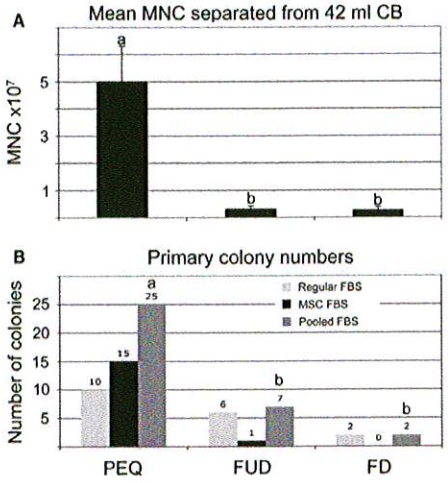

Figure 2. MNC concentrations and primary colony numbers. (A) Use of PEQ yielded significantly higher concentrations of MNC than methods of undiluted and diluted blood loaded onto Ficoll-Paque PREMIUM (FUD and FD groups, respectively). (B) Significantly more colonies were noted in the PEQ group than with either FUD or FD groups. Solid light gray column, regular FBS; solid black column, MSC-qualified FBS; gray checked column, regular and MSC-qualified FBS combined. Statistical significant differences at the level of $P < 0.05$ *are indicated by different lower case letters.*

compared with the FUD and FD groups (Figure 2B). In the PEQ group, 25 colonies were noted, with 15 cultured in MSC-qualified FBS and 10 cultured in regular FBS. There were no significant differences in the number of colonies established in the FUD and FD groups of seven and two colonies, respectively. In the FUD and FD groups only one colony was noted in the MSC-qualified FBS culture group. The difference in colony numbers using different serum sources was insufficient for statistical comparisons. The importance of screening multiple FBS batches to determine which ones provide the best support for isolation of human bone marrow-derived MSC has been reported previously [3,4]. Further work is required to determine whether commercially available MSC-qualified FBS improves the isolation of equine MSC.

After combining the two serum groups, the isolation success of putative MSC per blood sample for each of the three different MNC separation methods was as follows: PEQ, 100% (5/5); FUD, 60% (3/5); FD, 20% (1/5). A 100% isolation success for putative CB MSC, as noted in the PEQ group, is remarkable. In a previous study, a protocol similar to the FUD group of this study was used. Interestingly, the isolation success in the FUD group of the current study was 60%, which was very similar to the 57% of the previous report [1]. Recent reports on human CB MSC isolation illustrate that consistent and reproducible isolation continues to be challenging, with less than 60% isolation success [5–7].

The overall PDT per day for 15 cell lines was 0.89 (SEM 0.1029). PDT for equine adipose tissue-derived MSCs (AT-MSCs) has been reported as 2:190.9 days for the first 10 population doublings [8]. The PDT for equine bone marrow-derived MSCs (BM-MSCs) has been reported as 1.490.22 days for passages after initial cell line establishment, and was associated with a prolonged PDT of 4.991.6 days [9]. The isolated CB-MSCs appears to be more proliferative than AT-MSCs and BM-MSCs, although different culture conditions complicate a direct comparison.

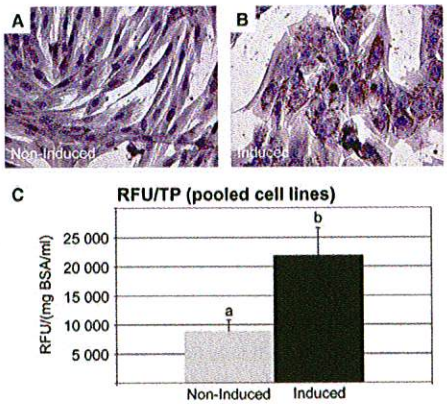

Figure 3. Assessment of adipogenesis. (A) Oil Red O staining of non-induced control cell cultures at day 10, magnification × 200. Few cells contain small Oil Red O-positive vacuoles. (B) Oil Red O staining of induced cell cultures on day 10, magnification × 200. Note the change of morphology toward large cuboidal cells and robust presence of Oil Red O vacuoles in all cells. The other two cell lines tested showed similar results. (C) Quantitative assessment of intracellular lipid content using the AdipoRed assay. The relative fluorescent unit (RFU) values were normalized against the total protein content of the culture wells. Induced cultures contained significantly more intracellular lipids than non-induced cultures ($P = 0.0006$).

All three tested cell lines could be induced to form pre-adipocytes and a statistically significant ($P = 0.0006$) increase in adipogenesis was measured in induced cultures compared with non-induced cultures using the quantitative AdipoRed assay (Figure 3). All three cell lines tested could also be induced toward the osteogenic cell lineage, as demonstrated by marked Alizarin Red S-positive calcium deposits in the induced cultures and a significant quantitative increase in alkaline phosphatase activity as well as calcium concentration in induced cultures compared with non-induced cultures (Figure 4). One in three tested cell lines showed robust chondrogenic differentiation, as demonstrated by an increase in pellet size over time as a result of production of extracellular matrix (ECM) and evidenced by the positive stain of glycosaminoglycans by Alcian Blue and Safranin O together with hyaline-like cartilage morphology of lacunae containing the chondrocyte (Figure 5). The reasons for the variable chondrogenic

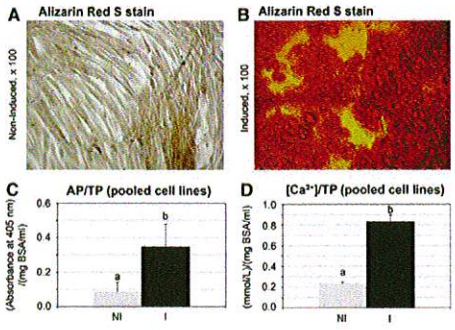

Figure 4. Assessment of osteogenesis. (A) Alizarin Red S (ARS) staining of non-induced control cell cultures at day 10, magnification × 100. (B) ARS staining of induced cell cultures at day 10, magnification × 100. Extensive ARS-positive ECM and no individual cells can be discerned. The other two cell lines tested showed similar results. (C) Quantitative assessment of alkaline phosphatase (AP) activity after 25 min of enzymatic activity. The AP absorbance values were normalized against the total protein (TP) content of the culture wells. Induced cultures (I) showed significantly more AP activity than non-induced cultures (NI) (P = 0.0012). (D) Quantitative assessment of calcium content per culture well. The calcium concentrations were normalized against the TP content measured in the AP wells. Induced cultures showed significantly more calcium deposits than non-induced cultures (P = 0.0006). Different letters above the bar graphs indicate statistically significant differences (P < 0.05).

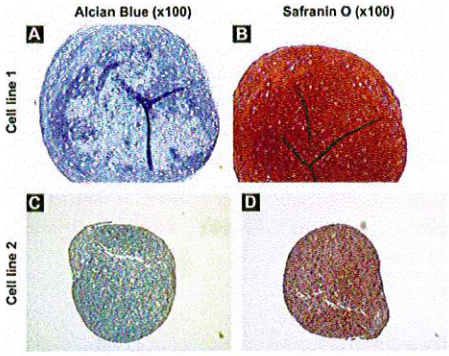

Figure 5. Assessment of chondrogenesis. One of three tested PEQ-derived putative MSC lines showed positive chondrogenesis (A, B). All images are at 100 times magnification with colors auto-adjusted using Adope® Photoshop® 7.0 software. Note the larger pellet size of (A) and (B) compared with the absent chondrogenesis of (C) and (D), reflecting deposition of ECM. Glycosaminoglycans, in the ECM, are positively stained with Alcian Blue and Safranin O. Chondrocytes within lacunae are also evident, consistent with a hyaline-like cartilage morphology. (C) and (D) are negative for the stains.

potential are undetermined. A variable chondrogenic potential of clonally expanded bovine BM MSC has been reported elsewhere [10]. A hierarchical loss of trilineage potency in human BM MSC has been advocated, with MSC losing adipogenic followed by chondrogenic and finally osteogenic potential loss [11]. In these differentiation experiments of human clonal BM MSC lines, cells with adipogenic and osteogenic potential, but not chondrogenic potential, were never identified [11]. More detailed investigation of chondrogenesis examining gene expression of chondrogenic markers could help determine whether the apparent chondrogenic response is truly absent.

Acknowledgements

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donated by BioE. BioE also markets similar products for human use: PrepaCyte®-CB and PrepaCyte®-WBC. PrepaCyte®-CB is a clinical product intended for processing human cord blood. PrepaCyte®-WBC, for research use only, is intended for processing human cord blood, peripheral blood or BM.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Koch TG, Heerkens T, Thomsen PD, Betts DH. Isolation of mesenchymal stem cells from equine umbilical cord blood. *BMC Biotechnol.* 2007;7(1):26.

2 Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res.* 1998;238(1):265–72.

3 Lennon DP, Caplan AI. Isolation of human marrow-derived mesenchymal stem cells. *Exp Hematol* 2006;34(11):1604–5.

4 Lennon DP, Haynesworth SE, Bruder SP, Jaiswal N, Caplan A. Human and animal mesenchymal progenitor cells from bone marrow: identification of serum for optimal selection and proliferation. *In Vitro Cell Dev Biol Anim.* 1996;32:602–11.

5 Rebelatto CK, Aguiar AM, Moretao MP, Senegaglia AC, Hansen P, Barchiki F, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp Biol Med.* 2008;233(7):901–13.

6 Cetrulo CL Jr. Cord-blood mesenchymal stem cells and tissue engineering. *Stem Cell Rev* 2006;2(2):163–8.

7 Reinisch A, Bartmann C, Rohde E, Schallmoser K, Bjelic-Radisic V, Lanzer G, *et al.* Humanized system to propagate cord blood-derived multipotent mesenchymal stromal cells for clinical application. *Regen Med* 2007;2(4):371–82.

8 Vidal MA, Kilroy GE, Lopez MJ, Johnson JR, Moore RM, Gimble JM. Characterization of equine adipose tissue-derived stromal cells: adipogenic and osteogenic capacity and comparison with bone marrow-derived mesenchymal stromal cells. *Vet Surg* 2007;36(7):613–22.

9 Vidal MA, Kilroy GE, Johnson JR, Lopez MJ, Moore RM, Gimble JM. Cell growth characteristics and differentiation frequency of adherent equine bone marrow-derived mesenchymal stromal cells: adipogenic and osteogenic capacity. *Vet Surg* 2006;35(7):601–10.

10 Jones EA, Crawford A, English A, Henshaw K, Mundy J, Corscadden D, *et al.* Synovial fluid mesenchymal stem cells in health and early osteoarthritis: detection and functional evaluation at the single-cell level. *Arth Rheum* 2008;58(6):1731–40.

11 Muraglia A, Cancedda R, Quarto R. Clonal mesenchymal progenitors from human bone marrow differentiate in vitro according to a hierarchical model. *J Cell Sci* 2000;113(7):1161–6.

Mammalian cell transfection*

*I've used this technique previously for MDA-MB-231BR eGFP cells at Stanford University in Stanford, CA, USA.

** The media used for the below cell line is DMEM with 10% FBS.

*** This will be repeated using the pDsRed Monomer-Hyg-N1 vector. The procedure for other vectors only differs in the selection agent used, please see step 7.

**MDA-MB-231BR eGFP cells

1. Seed 1.25 x 10^5 cells/well in a 6 well plate. For each vector used seed triplicate wells.
2. Allow cells to adhere overnight at 37C, 5% CO_2
3. The following day exchange media with 500 uL of fresh DMEM
4. In separate sterile Eppendorf tubes combine 500ng of plasmid, 100uL of OptiMEM media and 2 uL of Lipofectamine 2000. Make one tube for each well of cells to be transfected. Incubate at room temperature for 30 min.
5. Add full contents of each tube to a separate cell-seeded well. Let incubate overnight at 37C, 5% CO_2
6. After 24h split cells 1:10. Let incubate overnight at 37C, 5% CO_2
7. Add mammalian selection agent to each well. ***For pDsRed Monomer-Hyg-N1 use 125 ug/mL Hygromycin, for pcDNA3.1 or pEYFP use 500ug/mL Geneticin/Neomyocin respectively.
8. Grow cells under selection for 2-3 weeks, changing media every 3-4 days.
9. Once distinct drug-resistant colonies form, pick individual colonies into individual 24 well plate wells. Continue to grow under selection under a large clonal population is formed.
10. 4. To each well of a 24 well plate, add 20 000 cells, 8ug/mL of protamine sulfate (dissolved in PBS), the lentivirus suspension, and top off to a final volume of 1mL.
11. Keep track of cell growth; if necessary, move to larger tissue culture ware. Cells can be stored longterm in liquid nitrogen.

Plasmid Name	Gene Transfected	Results
(1) PCDNA3.1(+) 231PA	mms6 or magA	Gene expression
(2) PEYFP-C1	magA, YFP	Gene expression
(3) pDsRed monomer-Hyg-N1 (231Br)	mms6 or magA	Gene expression

Note: Currently we do not culture any of bacterial and modify cell lines with plasmids in our lab. All the modifications were done by our collaborators in their lab. The bacterial and plasmids are just stored in the freezer.

pcDNA3.1(+)
pcDNA3.1(-)

Catalog nos. V790-20 and V795-20, respectively

Version I
081401
28-0104

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Important Information

Contents

pcDNA3.1 is supplied as follows:

Catalog no.	Contents
V790-20	20 µg pcDNA3.1(+), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0
V795-20	20 µg pcDNA3.1(-), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Product Qualification

Each of the pcDNA3.1 vectors is qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pcDNA3.1(+)	*Nhe* I	5428
	Pst I	1356, 4072
	Sac I	109, 5319
pcDNA3.1(-)	*Nhe* I	5427
	Pst I	1363, 4064
	Sac I	169, 5258
pcDNA3.1/CAT	*Nhe* I	6217
	Pst I	2145, 4072
	Sac I	109, 6008

Methods

Overview

Introduction

pcDNA3.1(+) and pcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (-) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA3.1.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(+)

Below is the multiple cloning site for pcDNA3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(+) is available for downloading from our web site** (www.invitrogen.com) **or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(+), please refer to the **Appendix**, pages 10-11.

```
                                enhancer region (3′ end)
689  CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG

                                    CAAT                              TATA
749  TAACAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT

          3' end of hCMV            putative transcriptional start
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC

   T7 promoter/primer binding site          Nhe I      Pme I  Afl II Hind III  Asp718 I  Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC

   BamH I                        BstX I*  EcoR I        EcoR V        BstX I*   Not I     Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG

      Xba I          Apa I  Pme I                  pcDNA3.1/BGH reverse priming site
989  AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG

                BGH poly (A) site
1109 TCCTTTCCTA ATAAAATGAG GAAATTGCAT
```

*Please note that there are two *BstX* I sites in the polylinker.

continued on next page

Cloning into pcDNA3.1, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F′, DH5α™-T1^R, TOP10) and select transformants on LB plates containing 50 to 100 µg/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 µg aliquots.

Preparing a Glycerol Stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
- Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

Creation of Stable Cell Lines

Introduction

The pcDNA3.1(+) and pcDNA3.1(-) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

Geneticin® Selective Antibiotic

Geneticin® Selective Antibiotic blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® Selective Antibiotic is available from Invitrogen (Catalog no. 10486-025). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 800 µg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (see below). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take up to 3 weeks of growth in selective media.

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your gene of interest from pcDNA3.1, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. We recommend that you test a range of concentrations to ensure that you determine the minimum concentration necessary for your host cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Geneticin® (0, 50, 100, 200, 400, 600, 800 µg/ml Geneticin®).
3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Geneticin® that prevents growth within 2-3 weeks after addition of Geneticin®.

continued on next page

Creation of Stable Cell Lines, continued

Selection of Stable Integrants

Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

1. Transfect your mammalian host cell line with your pcDNA3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA3.1/CAT plasmid as a positive control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
4. Feed the cells with selective medium every 3-4 days until Geneticin®-resistant foci can be identified.
5. Pick and expand colonies in 96- or 48-well plates.

pcDNA3.1 Vectors, continued

Features of pcDNA3.1(+) and pcDNA3.1(-)

pcDNA3.1(+) (5428 bp) and pcDNA3.1(-) (5427 bp) contain the following elements. All features have been functionally tested.

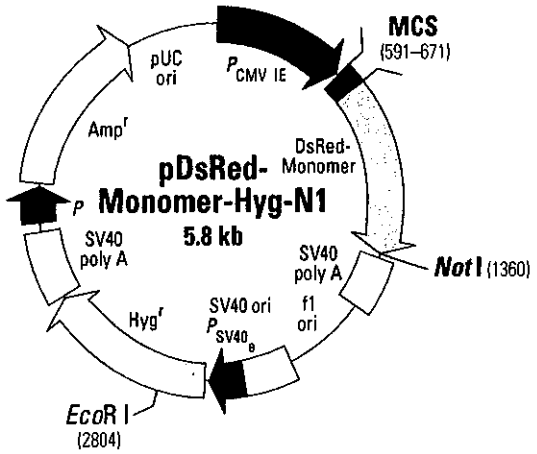
Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in forward or reverse orientation	Allows insertion of your gene and facilitates cloning
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*

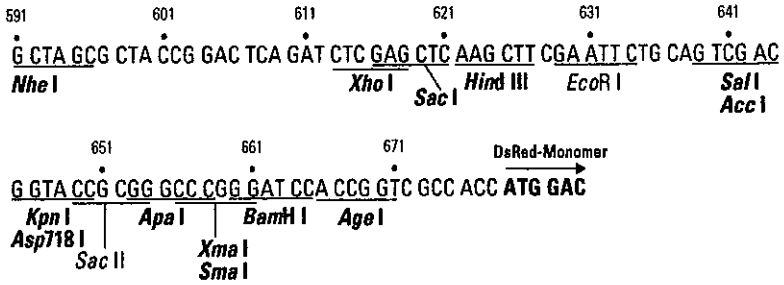
Vector Information

pDsRed-Monomer-Hyg-N1 Vector Information

PT3843-5

Cat. No. 632494





Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer-Hyg-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed-Monomer stop codon. NOTE: The *Xba* I and *Bcl* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Description

pDsRed-Monomer-Hyg-N1 is a mammalian expression vector that encodes DsRed-Monomer (DsRed.M1), a monomeric mutant derived from the tetrameric *Discosoma sp.* red fluorescent protein DsRed (1). DsRed-Monomer contains forty-five amino acid substitutions (listed on page 2). When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection (DsRed-Monomer excitation and emission maxima = 557 nm and 592 nm, respectively). The DsRed-Monomer coding sequence is human codon-optimized for high expression in mammalian cells (2).

DsRed-Monomer is well suited for use as a fusion tag. The multiple cloning site (MCS) in pDsRed-Monomer-Hyg-N1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed-Monomer coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed-Monomer if they are in the same reading frame as DsRed-Monomer and there are no intervening stop codons. A Kozak consensus sequence is located immediately upstream of the DsRed-Monomer gene to enhance translational efficiency in eukaryotic systems (3). SV40 polyadenylation signals downstream of the DsRed-Monomer gene direct proper processing of the 3' end of the DsRed-Monomer mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A hygromycin resistance cassette (Hyg^r)—consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals—allows stably transfected eukaryotic cells to be selected using hygromycin. A bacterial promoter-resistance gene cassette confers ampicillin resistance in *E. coli*.

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

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

GTG→TGC (Val-175 to Cys) mutation: 1201–1203
GAG→GAC (Glu-176 to Asp) mutation: 1204–1206
TCC→ACC (Ser-179 to Thr) mutation: 1213–1215
ATC→GTG (Ile-180 to Val) mutation: 1216–1218
ATG→AAG (Met-182 to Lys) mutation: 1222–1224
TAC→AAC (Tyr-192 to Asn) mutation: 1252–1254
TAC→CAC (Tyr-193 to His) mutation: 1255–1257
TCC→AAC (Ser-203 to Asn) mutation: 1285–1287
ATC→GTG (Ile-210 to Val) mutation: 1306–1308
CGC→CAC (Arg-216 to His) mutation: 1324–1326
ACC→GCC (Thr-217 to Ala) mutation: 1327–1329
GGC→GCC (Gly-219 to Ala) mutation: 1333–1335
CAC→TCC (His-222 to Ser) mutation: 1342–1344
CTG→GGC (Leu-223 to Gly) mutation: 1345–1347
TTC→TCC (Phe-224 to Ser) mutation: 1348–1350
CTG→CAG (Leu-225 to Gln) mutation: 1351–1353

- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1510–1515 & 1539–1544; mRNA 3' ends: 1548 & 1560
- f1 single-strand DNA origin: 1607–2062 (Packages the noncoding strand of DsRed-Monomer)
- SV40 origin of replication: 2403–2538
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2236–2307 & 2308–2379
  21-bp repeats: 2383–2403, 2404–2424 & 2426–2446
  Early promoter element: 2459–2465
  Major transcription start points: 2455, 2493, 2499 & 2504
- Hygromycin resistance gene:
  Start codon (ATG): 2560–2562; stop codon: 3583–3585
- SV40 early mRNA polyadenylation signal: 3732–3737 & 3761–3766; mRNA 3' ends: 3770 & 3782
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 3932–3937; –10 region: 3955–3960
- Ampicillin resistance gene:
  Start codon (ATG): 4002–4004; stop codon: 4860–4862
- pUC plasmid replication origin: 5025–5668

Sequencing primer location

- DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAACTGGGGGGACAG-3'): 879–859

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 the JM109 or XL1-Blue strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and emission maxima of DsRed-Monomer

- Excitation maximum = 557 nm
- Emission maximum = 592 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

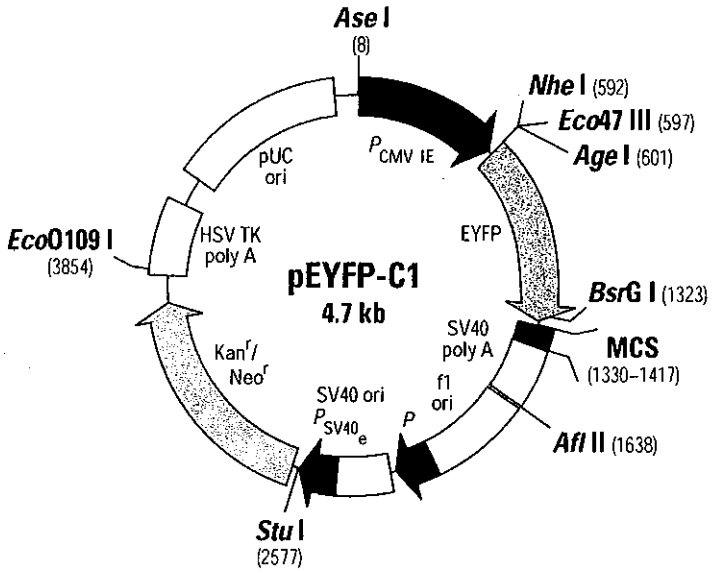
Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.

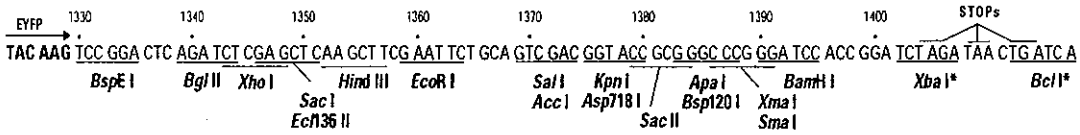
Vector Information

pEYFP-C1 Vector Information

PT3176-5

Catalog #6005-1





Restriction map and multiple cloning site (MCS) of pEYFP-C1. All restriction sites are shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-C1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513-nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-C1 is between the EYFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening in-frame stop codons. EYFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neor) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neor expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The recombinant EYFP vector can be

(PR29944; published 03 October 2002)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

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

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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Spin Infection

** This was done using the pCCL-EGFP-minCMV-hPGK-Luc lentiviral vector

*** Media used: PC3M cells used RPMI 1640 with 10% FBS, L-glutamine, Pen-Strep, 1x HEPES, and non-essential amino acids.

PC3M cells

1. Make sure the cells are growing nicely in log phase prior to transduction.
2. 2 days prior to transduction, seed a T25 flask approximately 1:4 from an 80% confluent flask.
3. On the day of transduction, lift the cells and count.
4. To each well of a 24 well plate, add 20 000 cells, 8ug/mL of protamine sulfate (dissolved in PBS), the lentivirus suspension, and top off to a final volume of 1mL.

1. The amount of lentivirus suspension to use depends on the multiplicity of infection you intend to go with. I used an MOI of 5 and got adequate levels of transductants.

1. The MOI refers to the number of transducing units per every cell. So an MOI of 5 with 20 000 cells meant 100 000TU

5. Spin down in the Beckman GS-15 centrifuge using the plate rotor

2. 2h30mins, 1000 x g, at room temperature, no brakes

1. I didn't try it with brakes

3. You'll need prewarmed media after this, so get some ready in the last hour of spinning.

6. Remove media and replace with a fresh 1mL of prewarmed media (per well)
7. Incubate at 37C, 5% CO2 for at least 48 hours to allow for integration and expression
8. Keep track of cell growth; if necessary, move to larger tissue culture ware

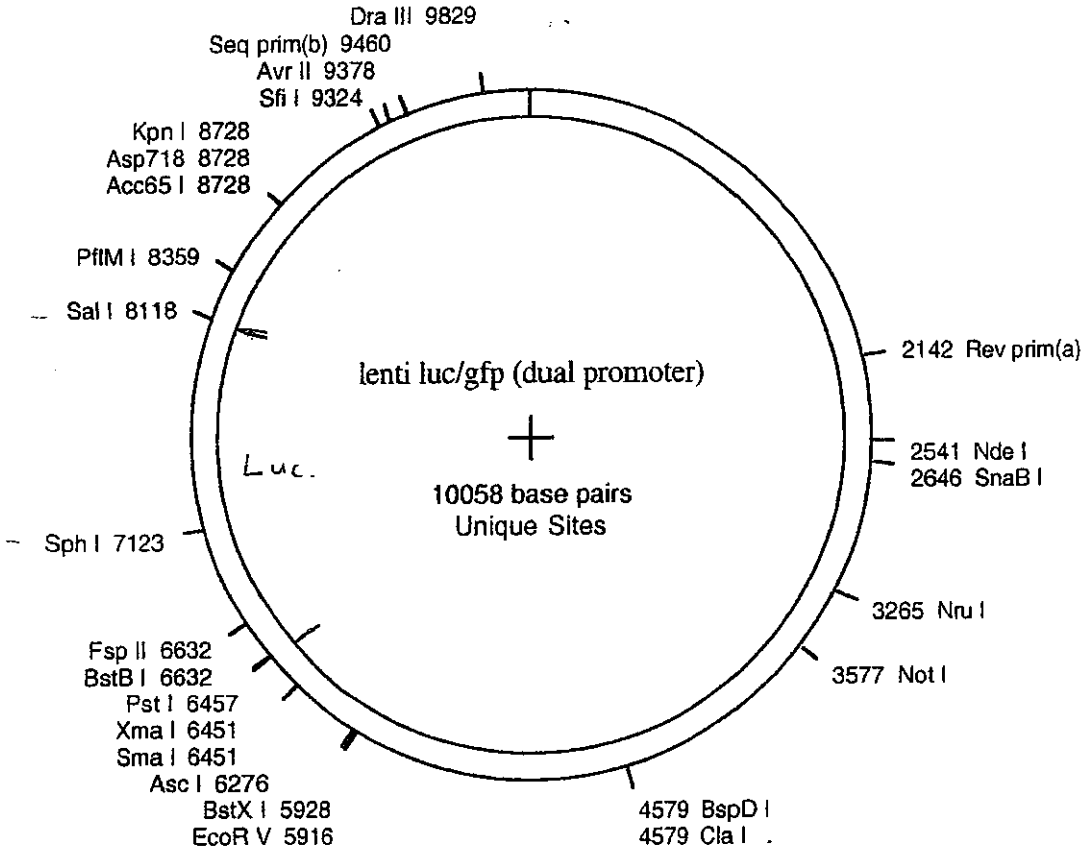
1. I didn't want to throw out any cells, so I lifted them with trypsin, and replated everything into a 6 well plate. Once that was confluent, it was moved to a T25, and lastly to a T75 as confluency dictated.

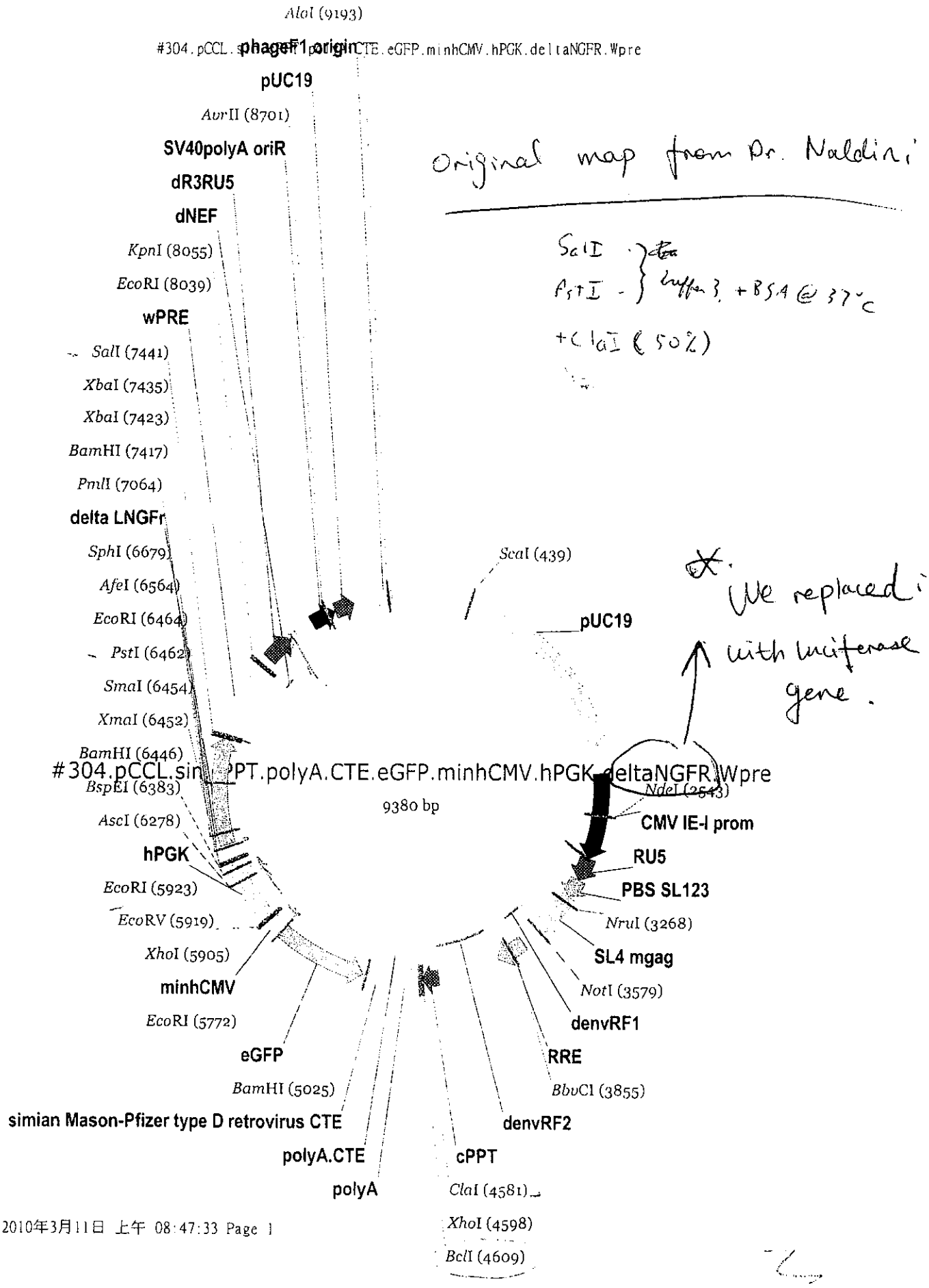
DNA Strider™ 1.2.1 ### Friday, March 31, 2006 10:07:19 AM

lenti luc/gfp (dual promoter) -> Graphic Map

DNA sequence 10058 b.p. caggtggcactt ... tttacaatttcc circular

#304.pCCL.sin.cPPT.polyA.CTE.eGFP.minhCMV.hPGK.luc.Wpre
PGKpromoter= 5928-6443 luc= 6464-8117 (PstI/SalI)
minhCMVpromoter=5786-5894 (reverse) eGFP=5051-5767 (reverse)

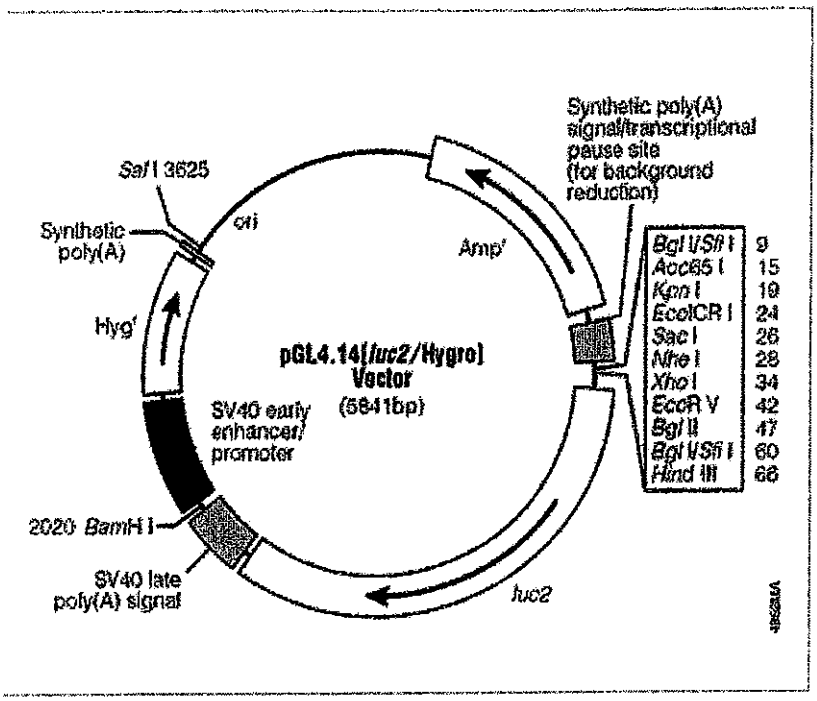




Original map from Dr. Naldini

SalI
PstI } Buffer 3 + BSA @ 37°C
+ClaI (50%)

* We replaced it with luciferase gene.



Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

LIST BIOLOGICAL LABORATORIES, INC.

40 DIVISION STREET ▪ CAMPBELL ▪ CALIFORNIA 95008-6906 ▪ USA
408-866-6363 ▪ 800-726-3213 ▪ FAX 408-866-6364 ▪ EMAIL info@listlabs.com

Material Safety Data Sheet(s)

MATERIAL SAFETY DATA SHEET
Pertussis Toxin
Pertusis Toxin (Salt-Free)

Ingredients:

Each vial contains 50.0 µg of lyophilized pertussis toxin (islet-activating protein). Product 180 also contains 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride, when reconstituted with 0.5 ml water.

Health Hazard Data:

The LD_{50} in mice is 18 µg/kg i.p. There is no LD_{50} information for humans.

Emergency Procedures:

Pertussis toxin is degraded by the low pH in the gut and is not absorbed. If swallowing occurs, induce vomiting.

If skin pricking should occur, induce bleeding and flush with copious amounts of water.

If i.v. or i.m. injection should occur, consult a physician. Attempt to obtain hyperimmune globulin to pertussis from the CDC. In an adult immunized versus whooping cough, no long term ill effects are likely to result.

Handling:

Pertussis toxin, in spite of its name, is not considered hazardous. However, as with any biochemical, it should be handled by trained personnel using good laboratory technique. Observe the following practices when working with pertussis toxin: Special care should be taken when working in conjunction with hypodermic needles. Wear protective gloves, avoid contact with cuts or wounds, avoid inhalation, do not mouth pipet, and flush thoroughly any area of the body that comes in contact with this product. Only individuals who were immunized in childhood against whooping cough should work with this product. This product is intended for research purposes only.

Stability:

Stable for months when stored at 4°C. Do not freeze.

Deactivation:

Boil at 100°C for 15 to 30 minutes.

Hazard Pertussis Toxin Study Information and maintaining records standard operation procedures

Pertussis Toxin purchased at Celdarlane Laboratories limited Company (Canadian distributor). Procedures of working with it must be performed in a complete containment level 2 Lab. It must have its usage recorded diligently. Quantities used, and for which approved animals are required details. Records must be filled out in ink, with no ditto marks, with one line per injection. Records are kept in a blue bender on the filing cabinet.

Pertussis Toxin must be monitored. Details about its specific usage can be found in the animal use protocols, and designated projects/animal information can be found in the file for the exemption located in the filing cabinet.

Pertussis Toxin that must be kept in a locked 4-degree fridge drawer at all times unless it is in use. Dilutions are always required. For example, It is diluted from 50ug/0.5ml to 200ng/0.2ml for each mouse use. A small aliquot of a dilution should be made when required. A designated individual such as the laboratory supervisor should make dilutions. No other member of the lab, besides the principle investigator, should be permitted to make dilutions or to withdraw from the stock bottle of pertussis toxin in fear of misuse or contamination. After the experiment, the remaining dilution should be discarded in the sharps container. This should be recorded in the records. Also, it is important to emphasize that whatever amount of pertussis toxin that is taken (based on weight), that amount must be recorded. Approximations should be avoided, as there will be a discrepancy in the amount left in the vial and what is indicated in the records.

New students or those who have proven themselves untrustworthy should not be allowed to draw up controlled drugs. They should plan ahead of time and ask the lab supervisor or another more senior person in the lab to draw the drugs for them to use until they have shown to be trustworthy.

Hazard Pertussis Toxin Study Information

Protocol Number 2009-*042*

Investigator Dr Paul Foster

Primary Research Tech: 1st Contact: Yuhua Chen Work: 24133 Home: 529 472-8279

Housing Room 6026

Hazard: Pertussis Toxin, injected IP at day 0 and day 2, with excretion for two days post injection

Transmission Route All Routes– Inhalation/Skin/Eyes/Ingestion/Injection
PPE: Normal Inclusionary PPE- Tyvek, bonnet, Niosh respirator,, double gloves, booties (put on as you go in from anteroom, off as your step back in to anteroom to leave)

Biology of the Agent: MSDS attached

Health Status of Animals on Study: Conventional, require non sterile micro isolators

Treatment for Exposure to agent? Refer to MSDS on reverse side of this sheet

Disinfectant(s) of choice: N/A, therefore will use Virkon for floor mopping, cage dunking and decontaminating material out of the room

Animal Room Biosafety Recommendations: PPE as stated above. All procedures with animals in the Class 2 Biological safety cabinet set up as per usual for barrier work.

> **Animal Care staff**: Scrape out dirty bedding with spatula in hood (and decon out of room for incineration) Animal bedding treated as hazardous waste and incinerated Dirty cages and water bottles should be dunked out of the room with Virkon.
>
> **Research staff:** Will bring mice up from conventional area; change into clean cages prior to injections. They will change mice to clean cages in the BSC ,prior to removal back to conventional . Minimum time to allow for excretion of toxin to stop is day 5 post injection

Accidental exposure procedures: Refer to MSDS on reverse side of this instruction sheet

LIST BIOLOGICAL LABORATORIES, INC.
540 DIVISION STREET ▪ CAMPBELL ▪ CALIFORNIA 95008-6906 ▪ USA
408-866-6363 ▪ 800-726-3213 ▪ FAX 408-866-6364 ▪ EMAIL info@listlabs.com
WEBSITE www.listlabs.com

PERTUSSIS TOXIN
(ISLET-ACTIVATING PROTEIN)

Pertussis toxin is the major protein toxin produced by virulent strains of ***Bordetella pertussis***, the organism that causes whooping cough.[1] As revealed by poly-acrylamide gel electrophoresis, the purified protein consists of five dissimilar subunits: S-1 (molecular weight 28,000 daltons), S-2 (23,000), S-3 (22,000), S-4 (11,700) and S-5 (9,300), in a molar ratio of 1:1:1:2:1. S-1 (A protomer) is responsible for the enzymic activity of the toxin. Together, S-2, S-3, S-4 and S-5 comprise the B oligomer, responsible for binding the toxin to the cell surface.[2]

Pertussis toxin elicits a variety of physiological responses, including induction of lymphocytosis, stimulation of insulin secretion, and sensitization to histamine. It has thus been variously referred to in the literature as lymphocytosis-promotingfactor,[3,4] islet-activating protein,[5] histamine-sensitizing factor [6] and pertussigen.[7] The toxin also acts as a hemagglutinin,[8] and serves as a protective antigen in mice against challenge with *B. pertussis*.[3,9,10,11] Further, treatment of Chinese hamster ovary cells with purified toxin results in a unique clustered growth pattern which provides a useful *in vitro* assay for the toxin.[12]

Pertussis toxin has been found to catalyze the ADP-ribosylation of the G_i regulatory component of adenylate cyclase,[13,14] and it is this action that is believed to be responsible for the various physiological and cellular effects of the toxin. Studies with rat heart cells,[15] pancreatic islets[16,17] and G6 glioma cells[18] have shown that treatment with pertussis toxin potentiates cyclic AMP accumulation in response to normal stimuli. Further, cells treated with the toxin fail to respond to agents that normally block cyclic AMP accumulation.[19] Thus, pertussis toxin has become a valuable tool in the study of the regulation of adenylate cyclase. The action of pertussis toxin on the G_i component of adenylate cyclase has also been found to inhibit various metabolic responses of neutrophils to chemotactic factors, implying a role for G_i in these functions as well.[20,21,22] Recently, it has been found that pertussis toxin catalyzes the ADP-ribosylation of transducin, a guanine nucleotide-binding regulatory protein that mediates activation of a retinal cyclic GMP-selective phosphodiesterase.[23,24]

Pertussis toxin from List Biological Laboratories is isolated from *Bordetella pertussis* by a modification of the method of Cowell *et al.*[25] This preparation is highly purified, migrating as five distinct bands as described by Tamura *et al.* [2] when run on 15% polyacrylamide SDS-urea gels according to the method of Laemmli.[26] It contains no detectable fimbrial hemagglutinin on gels, and no measurable *B. pertussis* adenylate cyclase activity when assayed by the method of Wolff *et al.*[27] in the absence or presence of 1 µmolar calmodulin. Each lot is tested for its activity in the CHO cell assay as described by Hewlett *et al.*[12]

Pertussis toxin is supplied in three formulations. The first is frozen in 50% glycerol, 0.05 M Tris, pH 7.5, 0.01 M glycine, 0.5 M NaCl. The second is lyophilized and when reconstituted with 0.5 ml of distilled water contains 50.0 µg of protein in 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride. Finally, pertussis toxin is also available lyophilized in a salt-free form. A detailed lot analysis documenting purity and biological activity plus complete instructions on reconstitution and storage accompany each shipment.

This product is intended for research purposes only and is not for use in humans. For further information, please contact List Biological Laboratories, Inc.

Ordering Information

Product No.	Description	Size
179A	Pertussis Toxin (frozen)	50.0 µg
179B	Persussis Toxin (frozen)	200.0 µg
180	Pertussis Toxin (lyophilized)	50.0 µg
181	Pertussis Toxin (lyophilized, salt-free)	50.0 µg

References

1. Pittman, M. (1979) *Rev. Infect. Dis.* **1**, 401-412.
2. Tamura, M., Nogomori, K., Murai, S., Yajima, M., Ito, K., Katada, T., Ui, M. and Ishi, S. (1982) *Biochem.* **21**, 5516-5522.
3. Sato, Y., Arai, H. and Suzuki, K. (1974) *Infect. Immun.* **9**, 801-810.
4. Morse, S.I. and Morse, J.H. (1976) *J. Exp. Med.* **143**, 1483-1502.
5. Yajima, M., Hosoda, K., Kanbayashi, Y., Nakamura, T., Nogimori, K., Mizushima, Y., Nakase, Y. and Ui, M. (1978) *J. Biochem. (Tokyo)* **83**, 295-303.
6. Muñoz, J.J. and Bergman, R.K. (1968) *Bacteriol. Rev.* **32**, 103-126.
7. Arai, H. and Muñoz, J.J. (1981) *Infect. Immun.* **31**, 495-499.
8. Irons, L.I. and MacLennan, A.P. (1979) *Biochim. Biophys. Acta* **580**, 175-185.
9. Muñoz, J.J., Arai, H. and Cole, R.L. (1981) *Infect. Immun.* **32**, 243-250.
10. Sato, Y., Izumiya, K., Sato, H., Cowell, J.L. and Manclark, C.R. (1981) *Infect. Immun.* **31**, 1223-1231.
11. Oda, M., Cowell, J.L., Burstyn, D.G. and Manclark, C.R. (1984) *J. Infect. Dis.* **150**, 823-833.
12. Hewlett, E.L., Sauer, K.T., Myers, G.A., Cowell, J.L. and Guerrant, R.L. (1983) *Infect. Immun.* **40**, 1198-1203.
13. Bokoch, G.M., Katada, T., Nortup, J.K., Hewlett, E.L. and Gilman, A.G. (1983) *J. Biol. Chem.* **258**, 2072-2075.
14. Codina, J., Hildebrandt, J., Iyengar, R., Birnbaumer, L., Sekura, R.D. and Manclark, C.R. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4276-4280.
15. Hazeki, O. and Ui, M. (1981) *J. Biol. Chem.* **256**, 2856-2862.
16. Katada, T. and Ui, M. (1979) *J. Biol. Chem.* **254**, 469-479.
17. Katada, T. and Ui, M. (1980) *J. Biol. Chem.* **255**, 9580-9588.
18. Katada, T., Takehito, A. and Ui, M. (1982) *J. Biol. Chem.* **257**, 3739-3746.
19. Katada, T. and Ui, M. (1981) *J. Biol. Chem.* **256**, 8310-8317.
20. Molski, T.F.P., Naccache, P.H., Marsh, M.L., Kermode, J., Becker, E.L. and Sha'afi, R.I. (1984) *Biochem. Biophys. Res. Comm.* **124**, 644-650.
21. Okajima, F. and Ui, M. (1984) *J. Biol. Chem.* **259**, 13863-13871.
22. Bokoch, G.M. and Gilman, A.G. (1984) *Cell* **39**, 301-308.
23. Manning, D.R. and Gilman, A.G. (1983) *J. Biol. Chem.* **258**, 7059-7063.
24. Manning, D.R., Fraser, B.A., Kahn, R.A. and Gilman, A.G. (1984) *J. Biol. Chem.* **259**, 749-756.
25. Cowell, J.L., Sato, Y., Sato, H., An der Lan, B. and Manclark, C.R. (1982) *Semin. Infect. Dis.* **4**, 371-379.
26. Laemmli, U.K. (1970) *Nature* **227**, 680-685.
27. Wolff, J., Cook, G.H., Goldhammer, A.R. and Berkowitz, S.A. (1980) *Proc. Natl. Acad. Sci.* U.S.A. **77**, 3841-3844.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Pertussis toxin
Proposed Use Dose:	0.2 µg
Proposed Storage Dose:	70 µg
LD_{50} (species):	18 µg

Calculation:

18 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 900

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 90

Comments/Recommendations:

New Info

To Whom It May Concern:

Please find enclosed a complete Biological Agents Registry Form. Attached you will also find the documents of biohazards and use protocols.

An issue I would like you to know that is about Dr. Foster's Postdoctoral Gabrielle Siegers. Gabrielle's contract is ending, but she is still in lab to complete her works untill end of Feb. Also, her cells still are stored in our freezer. So I keep all of her cell lines on the biohazard registry form for now. Her works will be performed in the culture facility Rm# 2222 in RRI 2nd floor. The documents and protocols of blood works are attached.

If you have any further questions regarding this application, please do not hesitate to contact me.

Sincerely,

Yuhua Chen.

Gabrielle Siegers' Cell Lines

Biosafety Level 1

K562
EM-2
TMD2
L363

Biosafety Level 2 (integrated viruses or primary cells)

MEC-1
K562eGFPluc
EM-2eGFPluc
RAJI
RAJIeGFPluc
LCLs
Primary Cells
Therapy mouse EM-2eGFPluc (from bone marrow)
Peripheral blood mononuclear cells (PBMCs)
- healthy donors and patient samples (Finland)
Gamma delta T cells (GDTCs)
Gamma delta T cell clones – healthy donors only

Public Health Agency of Canada | Agence de la santé publique du Canada

Permit no.-Permis no.

P-11-0060

Permit to import human pathogen(s) | **Permis d'importation d'agent(s) anthropopathogène(s)**

Under the authority of the Human Pathogens Importation Regulations. | Sous le régime du Règlement sur l'importation des agents anthropopathogènes.

Importer-Name, address and postal code - **Importateur**-Nom, adresse et code

University of Western Ontario
Department of Microbiology and Immunology
Robarts Research Institute
100 Perth Drive
London, Ontario, N6A 5K8

Facsimile-Télécopieur: 519-931-5224

Telephone no.- No. de téléphone: 519-663-5777 x 24086

Attn: Gabrielle Siegers

Supplier-Name and address - **Fournisseur**-Nom et adresse

Helsinki University Central Hospital
P.O. Box 700
Helsinki, FI-00029, Finland

Name(s) of Port(s) of Entry- To Clear Customs at Port(s) of entry
Nom(s) de(s) point(s) d'entrée-Dédouanement au(x) point(s) d'entrée

Various

Description of Pathogen(s)-For the importation of - **Description de(s) agent(s) anthropopathogène(s)**-Pour l'importation de

Human samples that may contain Risk Group 2 human pathogens.

On the following terms and conditions as marked:-Selon les conditions indiquées:

1. Work involving any of the imported material shall be limited to *in vitro* laboratory studies.	■	Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire *in vitro*.
2. Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.	■	Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.
3. All animals exposed to infection by any of the imported material shall be so exposed and held only in isolated insect-and rodent-proof facilities.	☐	Les animaux exposés à l'infection par la matière importée doivent y être exposés et être gardés uniquement dans des installations isolées à l'abri des insectes et des rongeurs.
4. All equipment, animal pens, cages, bedding, waste and other articles under the importer's control, that come in direct or indirect contact with any of the imported material, shall be sterilized by autoclaving or incinerated.	■	L'équipement, les enclos pour animaux, les cages, les litières, les déchets et tout autre article sous la responsabilité de l'importateur qui viennent en contact direct ou indirect avec la matière importée doivent être stérilisés par autoclavage ou incinérés.
5. Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.	☐	Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.
6. No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	■	La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis. AUCUNE AGENT ANTHROPOPATHOGÈNE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
7. On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.	☐	Au terme des travaux de l'importateur auxquels a servi l'agent anthropopathogène importé, celui-ci et tous ses dérivés doivent être
8. Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.	☐	On peut accompiir l'isolation, l'identification primaire, et/ou la manipulation au niveau de confinement 2 (exigences physiques) en utilisant les exigences opérationnelles de niveau de confinement 3.
9. NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	☐	AUCUNE MATIÈRE IMPORTÉE NE PEUT ÊTRE TRANSPORTÉE, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MISE EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
10. The Director must approve all new work with the imported material that requires an increase of containment from level 2.	☐	Tous nouveaux travaux avec la matière importée, qui requièrent une augmentation de niveau de confinement du niveau 2, exigeront l'approbation du Directeur.
11. No culturing of Risk Group 3 or 4 pathogens shall be done.	☐	Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise.

12. **This permit is valid only for:**
Le présent permis n'est valide que pour:

☐ a) a single entry into Canada **or**
une seule entrée au Canada **ou**

■ b) importations at intervals of
les importations effectuées à intervalles de

during the period beginning on
au cours de la période commençant le
2011-02-25

and ending on
et se terminant le
2012-03-07

Authorization-Signature of Director
Autorisation-Signature du Directeur

for Lise Murphy

Date 2011-02-25

Note: Transporting and otherwise dealing with imported material are subject to federal, provincial and municipal laws (if any), to the extent that, those laws apply in respect of that material.

Nota: Les opérations relatives à la matière importée, y compris le transport, sont assujetties aux lois fédérales, provinciales et aux règlements municipaux applicables.

Canada

Agency of Canada / publique du Canada

Centre for Emergency Preparedness and Response / Centre de mesures et d'interventions d'urgence

Protected when completed / Protéger une fois rempli

Application for permit to import human pathogen(s) / Demande de permis d'importation d'un (d')agent(s) anthropopathogène(s)

Under the authority of the Human Pathogens Importation Regulations. / Sous le régime du Règlement sur l'importation des agents anthropopathogènes.

For Office use only / À l'usage du bureau seulement
Permit no. - N° de permis

Forward copy to:
Office of Laboratory Security
100 Colonnade Road, Loc.: 6201A
OTTAWA ON K1A 0K9
Telephone: (613) 957-1779 Facsimile: (613) 941-0596

Envoyer la copie au :
Bureau de la sécurité des laboratoires
100, chemin Colonnade, Loc. : 6201A
OTTAWA ON K1A 0K9
Téléphone : (613) 957-1779 Télécopieur : (613) 941-0596

1. Applicant - Name, address and postal code / Demandeur - Nom, adresse, et code postal

Gabrielle M. Siegers, Foster Lab, Robarts Research Institute, PO Box 5015, 100 Perth Dr. Rm 3296, London, ON, N6A 5K8

Facsimile / Télécopieur: 519-931-5224

Telephone no. / N° de téléphone: 519-663-5777 ext 24086

2. Supplier - Name and address / Fournisseur - Nom et adresse

Anna Kreutzman, Hematology Research Unit, Biomedicum Helsinki, C428b, Department of Medicine, Helsinki University Central Hospital, P.O. Box 700, FI-00029 HUCH, Finland

3. Description of material comprising human pathogen (Including name of material, country of origin and human or animal source)
Description de la matière comprenant un agent anthropopathogène (notamment dénomination, pays d'origine et source humaine ou animale)

blood samples from human chronic myeloid leukemia patients in Finland

4. Mode of transportation / Mode de transport

courier (air/road)

5. Canadian port(s) of entry / Point(s) d'entrée au Canada (Note: Not more than one entry is permissible in the case of a human pathogen that belongs to risk group 3 or 4.) (Remarque : Une seule entrée est permise dans le cas d'un agent anthropopathogène des groupes de risque 3 ou 4.)

various

6. Quantity of material to be imported and in the case of a human pathogen belonging to risk group 2, any intervals at which, or period during which, the pathogens are to be imported.
Quantité de la matière à importer - Dans le cas d'un agent anthropopathogène du groupe de risque 2, toute intervalle ou période d'importation.

Approximately 600 ml human blood (60-90 ml/shipment at 4-6 week intervals) will be imported in a one-year period.

7. Description of applicant's facilities and equipment for handling material (Note: Appropriate containment is required: see the LABORATORY BIOSAFETY GUIDELINES as amended from time to time, established by Health Canada and the Medical Research Council of Canada).
Description des installations et de l'équipement du demandeur utilisés pour la manutention de la matière (Remarque : Confinement adéquat exigé : voir les LIGNES DIRECTRICES EN MATIÈRE DE BIOSÉCURITÉ EN LABORATOIRE, avec leurs modifications successives, établies par Santé Canada et le Conseil de recherche médicales du Canada).

Our laboratory meets Containment Level 2 requirements as per the Laboratory Biosafety Guidelines 3rd edition.

Additional information attached / Renseignements complémentaires ci-joint: ☐ Yes / Oui ☒ No / Non

8. Address of location where the human pathogen is to be used / Adresse du lieu où sera utilisé l'agent anthropopathogène

Robarts Research Institute,100 Perth Dr., London, ON, N6A 5K8

9. Method of treatment of material for the purposes of decontamination, sterilization and waste disposal
Méthode de traitement de la matière aux fins de décontamination, de stérilisation et de l'élimination des déchets

Liquid waste will be aspirated into a designated flask and treated with bleach prior to disposal.

10. Work objectives, proposed plan of work and additional pertinent information / Objectifs des travaux, plan de travail proposé et autres renseignements utiles

Gamma delta T cells (GDTc) constitute 2-5% of circulating lymphocytes in human blood. Since these cells elicit cytolytic responses against a variety of tumors in vitro and in vivo, immunotherapy using GDTc is currently under investigation in clinical trials. Using our GDTc isolation and expansion protocol we aim to: 1) assess whether GDTc can be expanded from the blood of chronic myeloid leukemia (CML) patients; 2) determine clonality of expanded GDTc; 3) assess functional capacity of expanded GDTc; 4) determine mechanisms of cytotoxicity of GDTc against CML cell lines and autologous tumours; and 5) assess iron oxide labeling of expanded GDTc for single cell imaging via MRI.

11. **Applicant**
I undertake that the material comprising the human pathogen will, in the event of its importation, be used in accordance with such terms and conditions as may be specified in the permit, and I certify that the facilities in which the material will, in that event, be manipulated and stored meet the following containment level;

Demandeur
Je m'engage à ce que la matière comprenant l'agent anthropopathogène, dans l'éventualité de son importation, soit utilisée conformément aux conditions du permis d'importation et j'atteste que les installations dans lesquelles cette matière sera manipulée et entreposée satisfont aux exigences du niveau de confinement suivant;

Containment level (Check one block only) / Niveau de confinement (Ne cocher qu'une seule case)

☐ 1 ☒ 2 ☐ 3 ☐ 4

Signature of applicant - Signature du demandeur: Gabrielle M Siegers

Date: 12 Jan 2011

*Note:
Physical containment levels and mechanical systems, operational protocols and laboratory waste disposal facilities are subject to verification as may be required by the Director.

*Remarque :
Les niveaux de confinement physique ainsi que les systèmes mécaniques, les protocoles opérationnels et les installations d'élimination des déchets de laboratoire sont soumis à une vérification si le Directeur l'exige.

Canada

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:	**TIB-202™** Order this Item	**Price:**	**$244.00**
Designations:	THP-1	**Depositors:**	S Tsuchiya
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	monocyte
Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;		
Cellular Products:	lysozyme [58053]		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
	Related Cell Culture Products		
Applications:	transfection host(technology from amaxa Roche FuGENE® Transfection Reagents)		
Receptors:	complement (C3), expressed [58053] Fc, expressed		
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16		
Age:	1 year infant		
Gender:	male		
Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58052] Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C		

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EM-2

Cell line:	EM-2
Cell type:	human chronic myeloid leukemia in blast crisis
DSMZ no.:	ACC 135
Origin:	established from the bone marrow of a 5-year-old Caucasian girl with Philadelphia-chromosome positive chronic myeloid leukemia (CML) in second relapse in 1980 (after bone marrow transplantation day +28); sister cell line of EM-3 (DSM ACC 134)
References:	Raskind et al., Cancer Genet Cytogenet 25: 271-284 (1987), PubMed ID 2 target= blank>3030532 Keating, Baillieres Clin Haematol 1: 1021-1029 (1987), PubMed ID 2852 target= blank>3332852 review: Drexler et al., Leuk Res 24: 109-115 (2000), PubMed ID 10654445
Depositor:	Dr. A. Keating, Toronto General Hospital, Ontario, Canada

DSMZ Cell Culture Data

Morphology:	single, round to oval cells in suspension
Medium:	90% RPMI 1640 + 10% FBS
Subculture:	maintain at 0.2-1.0 x 10^6 cells/ml; split ratio of 1:2 every 2-3 days; seed out at ca. 0.3-0.5 x 10^6 cells/ml
Incubation:	at 37 °C with 5% CO_2
Doubling time:	ca. 35-40 h
Harvest:	maximum density at about 1.0-1.5 x 10^6 cells/ml
Storage:	frozen with 70% medium, 20% FBS, 10% DMSO at about 6 x 10^6 cells/ampoule

DSMZ Scientific Data

Mycoplasma:	negative in DAPI, microbiological culture, RNA hybridization, PCR assays
Immunology:	CD3 -, CD4 +, CD13 +, CD14 -, CD15 +, CD19 -, CD33 +, CD34 -, HLA-DR -; image
Fingerprint:	same DNA profile as EM-3 using multiplex PCR of minisatellite markers
Species:	confirmed as human with IEF of AST, MDH, NP
Cytogenetics:	human hypertriploid karyotype with hypotetraploid sideline - 74(70-86)<3n>X, -X, -X, +3, +4, +6, +6, +6, +8, -9, +11, -14, -14, +15, +17, -19, +21, +22, +mar - der(5)t(5;?)(q13-15;?), der(9)t(9;22)(q34;q11), i(17q)x2 - in some cells up to three copies of der(9) are present - the karyotype overlaps that of EM-3 (DSM ACC 134) and corresponds with those reported for both EM-2 and EM-3
Molec. Genetics:	
Viruses:	ELISA: reverse transcriptase negative; PCR: EBV -, HBV -, HCV -, HHV-8 -, HIV -, HTLV-I/II -, SMRV -

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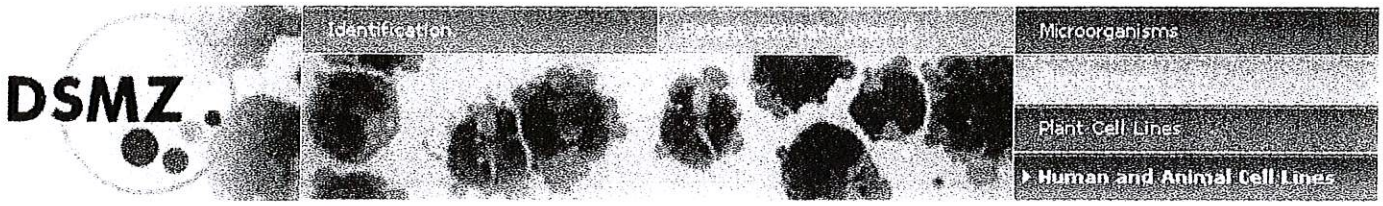

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MEC-1

Cell line:	MEC-1
Cell type:	human chronic B cell leukemia
DSMZ no.:	ACC 497
Origin:	established in 1993 from the peripheral blood of a 61-year-old Caucasian man with chronic B cell leukemia (B-CLL in prolymphocytoid transformation to B-PLL); serial sister cell line of MEC-2 (DSM ACC 500)
References:	Stacchini et al., Leuk Res 23: 127-136 (1999), PubMed ID 10071128
Depositor:	Prof. F. Caligaris-Cappio, Hospital Mauriziano Umberto, Turin, Italy

DSMZ Cell Culture Data

Morphology:	round to polymorphic cells growing in suspension, singly or partly in small aggregates, a few cells are slightly adherent
Medium:	90% Iscove's MDM + 10% FBS
Subculture:	split saturated culture 1:2 to 1:3 every 2-3 days; seed out at ca. 0.5 x 10^6 cells/ml; after thawing, during the first week cells should be kept with 20% FBS initially; culture in 24-well-plate is of advantage; maintain at about 0.5-2.0 x 10^6 cells/ml
Incubation:	at 37 °C with 5% CO_2
Doubling time:	ca. 40 hours
Harvest:	cell harvest of about 2.0 x 10^6 cells/ml
Storage:	frozen with 70% medium, 20% FBS, 10% DMSO at about 5 x 10^6 cells/ampoule

DSMZ Scientific Data

Mycoplasma:	contamination was eliminated with BM-Cyclin (tiamulin & minocycline), then negative in microbiological culture, RNA hybridization, PCR assays
Immunology:	CD3 -, CD10 -, CD13 -, CD19 +, CD20 +, CD34 -, CD37 +, cyCD79a +, CD80 +, CD138 -, HLA-DR +, sm/cyIgG -, sm/cyIgM +, sm/cykappa +, sm/cylambda -
Fingerprint:	same DNA profile as MEC-2 using multiplex PCR of minisatellite markers
Species:	confirmed as human by cytogenetics
Cytogenetics:	human near-diploid karyotype with 10% polyploidy - 46(44-47)<2n>XY, -2, +7, -12, +1-2mar, t(1;6)(q22-23;p21), add(7)(q11), der(10)(10pter->q22::?::2q11->qter), del(17)(p11) - small acf/mar present in most cells - resembles published karyotype
Molec. Genetics:	
Viruses:	PCR: EBV +, HBV -, HCV -, HIV -, HTLV-I/II -, SMRV -

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Cell Biology

ATCC® Number:	**CCL-86™** Order this Item	Price:	**$272.00**

Designations:	Raji
Depositors:	W Henle
Biosafety Level:	2 [CELLS CONTAIN HERPESVIRUS (EBV)]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Disease:** Burkitt's lymphoma **Cell Type:** B lymphocyte;
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.
Isolation:	**Isolation date:** 1963
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Resistance:	The cells are partially resistant to poliovirus and vesicular stomatitis viruses.
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 13 D16S539: 8,11 D5S818: 10,13 D7S820: 10 THO1: 6,7 TPOX: 8,13 vWA: 16,19
Cytogenetic Analysis:	Karyotype 100% stable within male diploid stemline of 46. Cells with 47 chromosomes frequently contained an extra "E" group chromosome.; There is 6% polyploidy and occasional disparity in the size of the homologs of the number 1 chromosome and the number 4 chromosome.
Age:	11 years

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22142: Pulvertaft JV. Cytology of Burkitt's tumour (African lymphoma). Lancet 1: 238-240, 1964. PubMed: 14086209
22169: Epstein MA, Barr YM. Characteristics and mode of growth of tissue culture strain (EB1) of human lymphoblasts from Burkitt's lymphoma. J. Natl. Cancer Inst. 34: 231-240, 1965. PubMed: 14293790
22550: Ohsugi Y, et al. Tumorigenicity of human malignant lymphoblasts: comparative study with unmanipulated nude mice, antilymphocyte serum-treated nude mice, and X-irradiated nude mice. J. Natl. Cancer Inst. 65: 715-718, 1980. PubMed: 6932523
22572: Moore PS, et al. Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. J. Virol. 70: 549-558, 1996. PubMed: 8523568
26253: Epstein MA, et al. Morphological and virological investigations on cultured Burkitt tumor lymphoblasts (strain Raji). J. Natl. Cancer Inst. 37: 547-559, 1966. PubMed: 4288580
26254: . . Trans. N.Y. Acad. Sci. 29: 61, 1966.
32395: Clark RA, et al. Tenascin supports lymphocyte rolling. J. Cell Biol. 137: 755-765, 1997. PubMed: 9151679
32448: Rich SA, et al. Purification, microresequencing, and immunolocalization of p36, a new interferon-alpha-induced protein that is associated with human lupus inclusions. J. Biol. Chem. 271: 1118-1126, 1996. PubMed: 8557639

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ATCC® Number:	**CCL-243™**	Order this Item	Price:	**$256.00**

Designations:	K-562
Depositors:	HT Holden
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	**suspension**
Organism:	***Homo sapiens* (human)**
Morphology:	**lymphoblast**
Source:	**Organ:** bone marrow **Disease:** chronic myelogenous leukemia (CML)
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)
Tumorigenic:	**Yes**
Antigen Expression:	**CD7 (25%)**
DNA Profile (STR):	Amelogenin: X **CSF1PO: 9,10** **D13S317: 8** **D16S539: 11,12** **D5S818: 11,12** **D7S820: 9,11** THO1: 9.3 TPOX: 8,9 vWA: 16
Cytogenetic Analysis:	**The stemline chromosome number is triploid with the 2S component occurring at 4.2%. Fifteen markers (M1 and M(15)) occurred in nearly all S metaphases. Spontaneous non-specific dicentrics occurred, but rarely. Unstable markers were also rarely seen. The X was disomic, and N9 was nullisomic.**

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Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 0 PGM1, 0 PGM3, 1
Age:	53 years
Gender:	female
Comments:	The continuous cell line K-562 was established by Lozzio and Lozzio from the pleural effusion of a 53-year-old female with chronic myelogenous leukemia in terminal blast crises. [22609] The cell population has been characterized as highly undifferentiated and of the granulocytic series. [26059] Studies conducted by Anderson, et al., on the surface membrane properties led to the conclusion that the K-562 was a human erythroleukemia line. [26060] The K-562 cell line has attained widespread use as a highly sensitive in vitro target for the natural killer assay. [1101] [48829] [48830] See Pross, et al. for a detailed analysis of the in vitro assay of NK cells including the mathematics of quantitation of NK cell activity. [48833] K-562 blasts are multipotential, hematopoietic malignant cells that spontaneously differentiate into recognizable progenitors of the erythrocytic, granulocytic and monocytic series. [26061] The effect of inducers on sublines derived from the original K-562 cell line have been reviewed by Koeffler and Golde. [867] Cultures from the ATCCstock have been shown to exhibit this sensitivity for assessing human natural killer activity. Karyological studies on various K-562 sublines have been classified into three groups (A,B,C) by Dimery, et al. [26063] The strain obtained by the ATCCmost closely resembles the B population. Occurrence of the Philadelphia chromosome, however, was of much lower frequency; none detected in 15 metaphases examined. The line is EBNA negative.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. 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

requires interaction between the promoter and intron. J. Biol. Chem. 271: 12387-12393, 1996. PubMed: 8647842
32704: Chan YJ, et al. Synergistic interactions between overlapping binding sites for the serum response factor and ELK-1 proteins mediate both basal enhancement and phorbol ester responsiveness of primate cytomegalovirus. J. Virol. 70: 8590-8605, 1996. PubMed: 8970984
33044: Nauseel WM, et al. Effect of the R569W missense mutation on the biosynthesis of myeloperoxidase. J. Biol. Chem. 271: 9546-9549, 1996. PubMed: 8621627
33174: Grune T, et al. Degradation of oxidized proteins in K562 human hematopoietic cells by proteasome. J. Biol. Chem. 271: 15504-15509, 1996. PubMed: 8663134
48829: Jondal M, Pross H. Surface markers on human b and t lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulations. Int. J. Cancer 15: 596-605, 1975. PubMed: 806545
48830: West WH, et al. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. J. Immunol. 118: 355-361, 1977. PubMed: 299761
48833: Pross HF, et al. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J. Clin. Immunol. 1: 51-63, 1981. PubMed: 7334070
61237: Chen TR. Modal karyotype of human leukemia cell line, K562 (ATCCCCL 243). Cancer Genet. Cytogenet. 17: 55-60, 1985. PubMed: 3857109
61327: Wu SQ, et al. Extensive amplification of bcr/abl fusion genes clustered on three marker chromosomes in human leukemic cell line K-56. Leukemia 9: 858-862, 1995. PubMed: 7769849

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GDTc cloning protocol (P. Fisch)

Protocol:

1) Isolate PBMCs from 20 ml blood.
2) FACS sort (and count) γδ T cells by positive selection directly into vial containing irradiated feeder cells* (1 x 10^6/ml) in medium, then mix well with fresh medium. The laser should be turned down as low as possible <100 mW.
3) Plate out at 1 cell/well (according to FACS count) into 96 well plates. Actual density will likely be 0.5 cell/well. Grow at 7% CO_2. Clones should come up after 9-12 days.
4) Wait another 2-3 days, then pick the clones, transferring them from 1 well into 6 into fresh medium with fresh feeders. Use only the inner 60 wells, thus you have 10 clones/plate.
5) Once they have grown a bit, test them for desired characteristics (cytotoxicity, FACS...) and then select them carefully. Once selected, plate only 1 clone/plate.
6) Freeze 2-3 vials/clone as early as possible. Once confluent (medium can get slightly yellowish), harvest plate almost entirely and freeze down in cold freezing medium. Transfer the remaining cells to a fresh plate with fresh medium and feeders.
7) Passage the cells once per week-10 days. When plating, seed at less than 2 x 10^6/plate and allow to grow up to 10 x 10^6.

* Feeder cells can be allogeneic. Freeze aliquots of PBMCs, thaw and then irradiate. For best results, use fresh feeders. Use 2 x 10^4 – 5 x 10^4 PBMCs/well and 1 x 10^4 LCLs. LCLs are very important for establishing clones; less yield is achieved with PBLs alone. Irradiate PBMCs at 5-10 Gey, LCLs at 30 Gey. If only using autologous PBMCs (no LCLs), then use 3-5x10^4 per well.

The protocol below describes how the LCL cell lines were made; I do not plan to generate more LCLs during my stay at Western. I will be using the immortalized lines (CS and WO) that were generated in Toronto and brought here. Thanks!

Generation of EBV-transformed B cell lines

Prepare 5ml of complete culture medium containing 1μg/ml Cyclosporin A. Separate mononuclear cells from a minimum of 5 ml of peripheral blood Centrifuge 5×10^6 PBMC for 5 minutes at 400 x g. Remove supernatant. Resuspend cells in 1.8 ml of complete culture medium containing 1μg Cyclosporin A. Add 100-200μl of the concentrated B95-8 virus supernatant to cell mixture. Mix gently. Place 200μl of the cell mixture in each of 5 wells of a 96-well flat-bottomed plate. Place 100μl of cell mixture into another 10 wells of the same 96 well flat bottom plate. Add an additional 100μl of CSA containing medium to the 10 wells containing 100μl of cell suspension. The final volume per well is 200μl. Fill the outside wells with sterile water. Place plate(s) in 37°C, 5% CO_2 incubator. Feed plate weekly: Remove plate(s) from incubator and aspirate 0.1 ml of media from each well of the 96-well flat-bottom plate. Add 0.1ml of fresh media to each well and return to incubator. After two weeks of incubation, if transformed cell clumps are expanding, combine cells from three wells of the 96-well plate into one well of a 24-well flat-bottomed plate. Repeat two more times to obtain a total of 3 wells in a 24-well plate. Retain remaining cells in 96-well plate as reserves. Incubate plate at 37°C for one week in a CO_2 incubator for further cell expansion. Once cells are clearly proliferating, remove cells from 24-well plate and place them into a 25 cm^2 vented flask with 5ml complete media plus 100μM of Acyclovir. Acyclovir is added to ensure that there will be no infectious virus present in the culture. Feed cells 1-2 times per week with complete media, adding 100μM Acyclovir each time. After approximately two weeks, freeze 5 vials with at least 2×10^6 LCLs. LCLs may be maintained in culture for a maximum of 9 months post initiation.

γδ T cell isolation and expansion protocol

Dec 2009

Materials

MACS buffer
AIMV medium + 5% heat inactivated hu serum
ConA
IL-2
IL-4
MACS antibody and beads
Ice
MACS column, magnet and holder
Eppis for counting
24 well plates (Falcon)

1. Isolate PBMCs from donors. Expected yield ~1.0 x 10^6 cells/ml
   COUNT:
2. Spin 5 min at 1800 rpm. Resuspend cells at 40µl/10^7 cells in cold MACS buffer (degassed) in a 50 ml conical.
   µl buffer used:
3. Remove 6µl for FACS analysis.
4. Add γδ TCR hapten antibody at 10µl/10^7 cells. Incubate in the fridge for 10 min.
   µl Ab used:
   incubation time:
5. Wash with 10 ml cold MACS buffer. Spin 5 min 1500 rpm 4 deg. Discard supernatant.
6. Resuspend cells at 30µl/10^7 cells in cold MACS buffer.
   µl buffer used:
7. Add anti-hapten-FITC antibody/beads at 20µl/10^7 cells. Incubate in the fridge for 15 min. µl Ab used:
   incubation time:
8. Wash with 10 ml cold MACS buffer. Spin 5 min 1500 rpm 4 deg.
9. Prime MACS column by inserting it into holder and carefully adding 500µl.
10. Discard supernatant and resuspend cell pellet in 500µl cold MACS buffer and then carefully add to column, by placing tip against the side of the column and allowing the cell suspension to run down the side.
11. Wash column with 3 x 500µl MACS buffer.
12. Add 3 ml medium to column. Remove column from magnetic holder and place into a fresh 15 ml conical. Insert plunger and press down to elute cells from the column.
13. Count cells, resuspend at 2.5 x 10^5 cells/ml and seed into 24-well plates (2ml/well). Use 1-2 ml for FACS analysis (wash in FACS buffer 1 x before antibody incubation).
14. Remove 75µl (15µl/sample) from MACS negative fraction for FACS analysis.

FACS Staining:

	preMACS	MACS+	MACS-
unstained	1	7	9
CD3 FITC 1:100	2	-	10
γδ TCR PE 1:10	3	-	11
αβ TCR PE 1:20	4	8	12
Vδ2 PE 1:100	5	-	
Vδ1 FITC 1:50	6		

Antibodies: 20-50 μl dilution/sample

Important notes:

50 ml conicals for isolation and antibody incubations
25-27 min primary antibody and 15 min secondary antibody incubations for MACS sorting
Con A 7-8 days (8 days better!)
remove all medium and replace with fresh at each feeding
spin down at 800rpm (sugg by Simone) ROOM TEMP
96 or 24 well-plates are better than 6-wells or 12-wells
12 and 6-wells are fine for passages beyond ConA and the first passage after ConA removal
expansion stops in T75 flasks

best seeding density 2.5-5 x 10E5 cells/ml
don't change med until med is apricot or yellowish
spin at RT

AIM-V medium (can probably use RPMI)
5% hu AB serum
10 ng/ml IL-2
10 ng/ml IL-4

first 6-8 days:
1μg/ml Concanavalin A

Keep the clones up to 4 weeks in culture after they begin to expand. You cannot say by vision if cells are clonal or polyclonal. As a matter of fact, gamma delta clones typically grow in a "flower-type" shape since the cells show active motility,particularly the acivated clones with high cytotoxic potential...They may however also grow as round colonies if they are longer in culture or if they tend "to be overgrown".

Thawing: Thaw 1 vial in fresh Iscoves Medium with 10% HS no cytokines. Centrifuge cells, then resuspend in complete medium with IL-2, irradiated feeders and PHA. Plate 60 wells on one plate. Paul never counted them after thawing. If it worked you should see growth with naked eyed within 1 to three days... If you have too many clones to thaw you can also plate on ½ plate (30 wells) and expand later once they grow. To improve conditions it helps taking fresh feeder cells and not frozen ones...