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
DH5α E.Coli strain	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.5L**	**Invitrogen**
	☐ 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: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

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

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

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-Iuc-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) 1	NCI (CRL-1435)	
(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
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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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Related Cell Culture Products

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 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
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Product Description

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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		

Related Links

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Cell Micrograph

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Related Cell Culture Products

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

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		

Related Links

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Cell Micrograph
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Frequently Asked Questions
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Related Cell Culture Products

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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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
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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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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.

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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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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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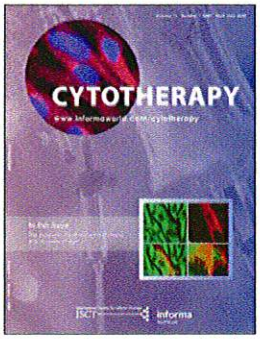

Cytotherapy
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Improved isolation protocol for equine cord blood-derived mesenchymal stromal cells

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Cytotherapy (2009) Vol. 11, No. 4, 443–447

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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

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DOI: 10.1080/14653240902887259

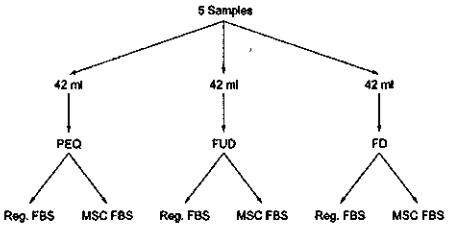

Figure 1. Study design. Each of five fresh CB samples was split into three groups of 42 mL and the nuclear or MNC fraction was isolated using the PEQ, FUD or FD protocol. Cell suspensions from each of the three separation groups were split further into two groups to allow assessment of two different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

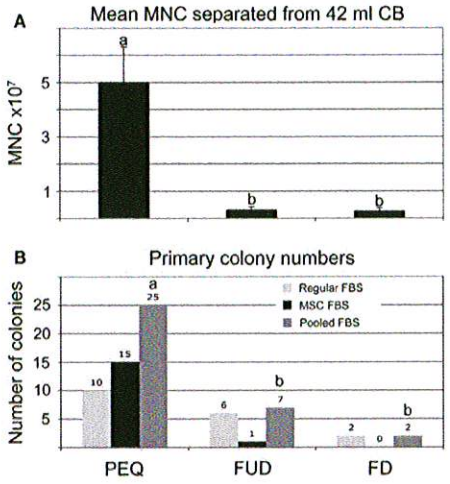

Figure 2. MNC concentrations and primary colony numbers. (A) Use of PEQ yielded significantly higher concentrations of MNC than methods of undiluted and diluted blood loaded onto Ficoll-Paque PREMIUM (FUD and FD groups, respectively). (B) Significantly more colonies were noted in the PEQ group than with 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.

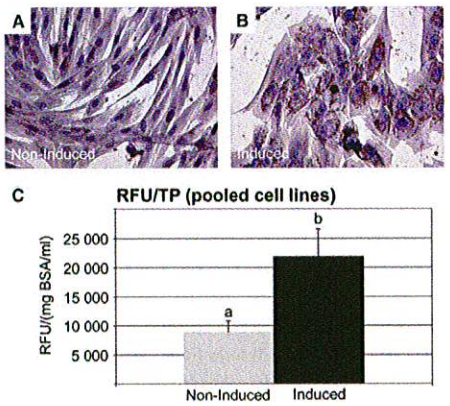

Figure 3. Assessment of adipogenesis. (A) Oil Red O staining of non-induced control cell cultures at day 10, magnification × 200. Few cells contain small Oil Red O-positive vacuoles. (B) Oil Red O staining of induced cell cultures on day 10, magnification × 200. Note the change of morphology toward large 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 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.

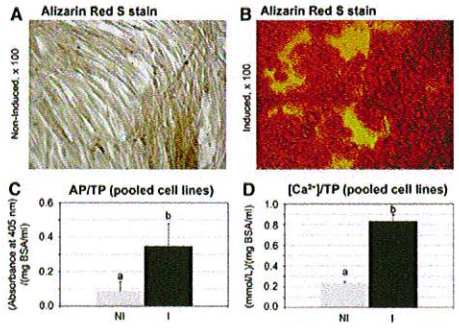

Figure 4. Assessment of osteogenesis. (A) Alizarin Red S (ARS) staining of non-induced control cell cultures at day 10, magnification × 100. (B) ARS staining of induced cell cultures at day 10, magnification × 100. Extensive ARS-positive ECM and no individual cells can be discerned. The other two cell 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 ($P < 0.05$).

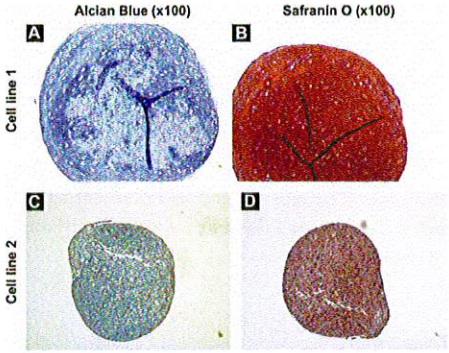

Figure 5. Assessment of chondrogenesis. One of three tested PEQ-derived putative MSC lines showed positive chondrogenesis (A, B). All images are at 100 times magnification with colors auto-adjusted using Adope® Photoshop® 7.0 software. Note the larger pellet size of (A) and (B) compared with the absent 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.

Acknowledgements

Daniel Gillis is acknowledged for statistical assistance. Windfield Farms, Oshawa, Ontario, Canada, is acknowledged for collection of cord blood.

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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.

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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

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

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 *Asp*718 I *Kpn* I

```
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
```

*Bam*H I *Bst*X I* *Eco*R I *Eco*R V *Bst*X 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 *Bst*X I sites in the polylinker.

continued on next page

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

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*

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

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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.

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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

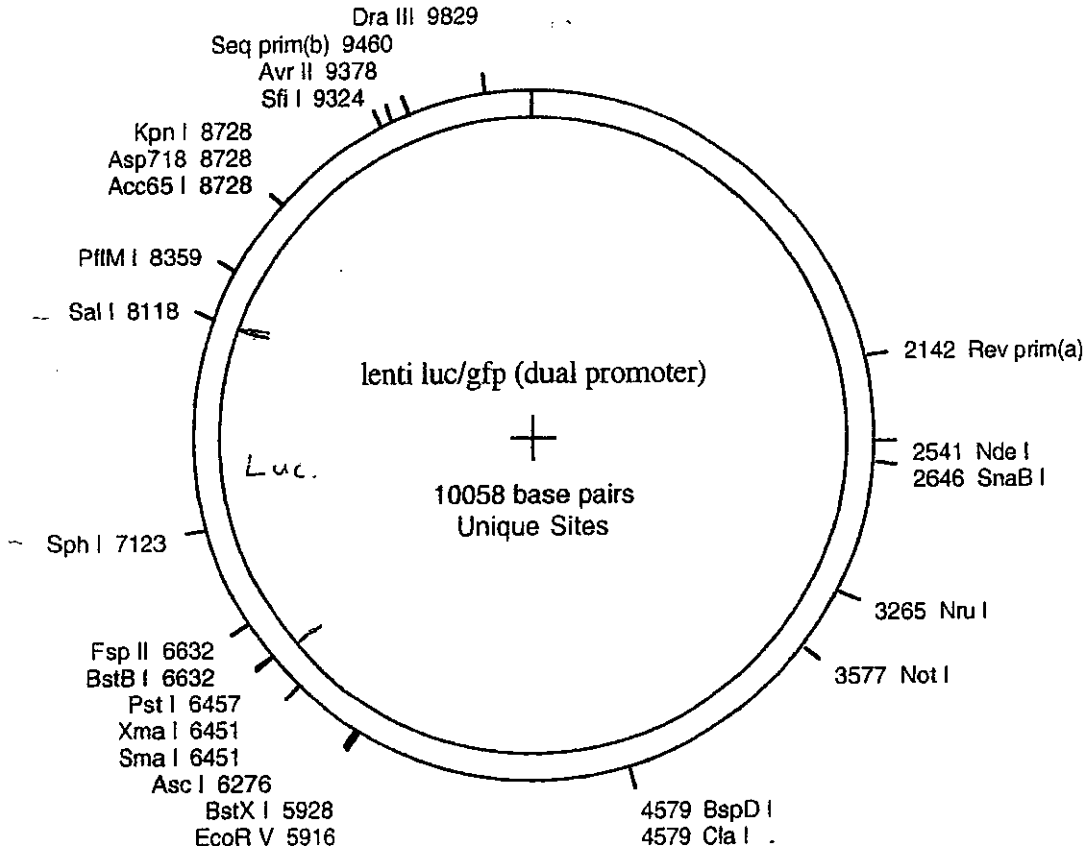
1. I didn't want to throw out any cells, so I lifted them with trypsin, and replated everything into a 6 well plate. Once that was confluent, it was moved to a T25, and lastly to a T75 as confluency dictated.

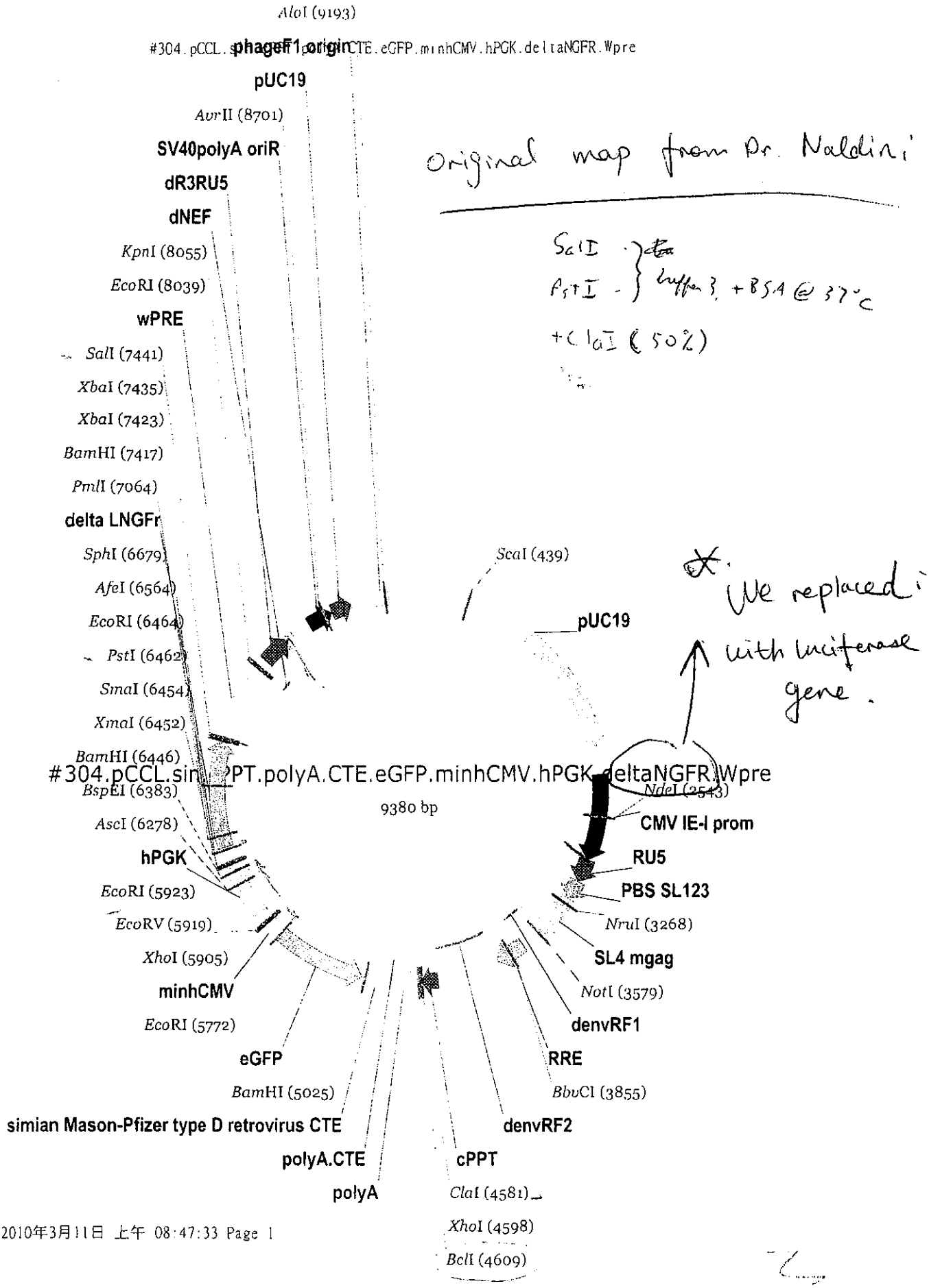
DNA Strider™ 1.2.1 ### Friday, March 31, 2006 10:07:19 AM

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.

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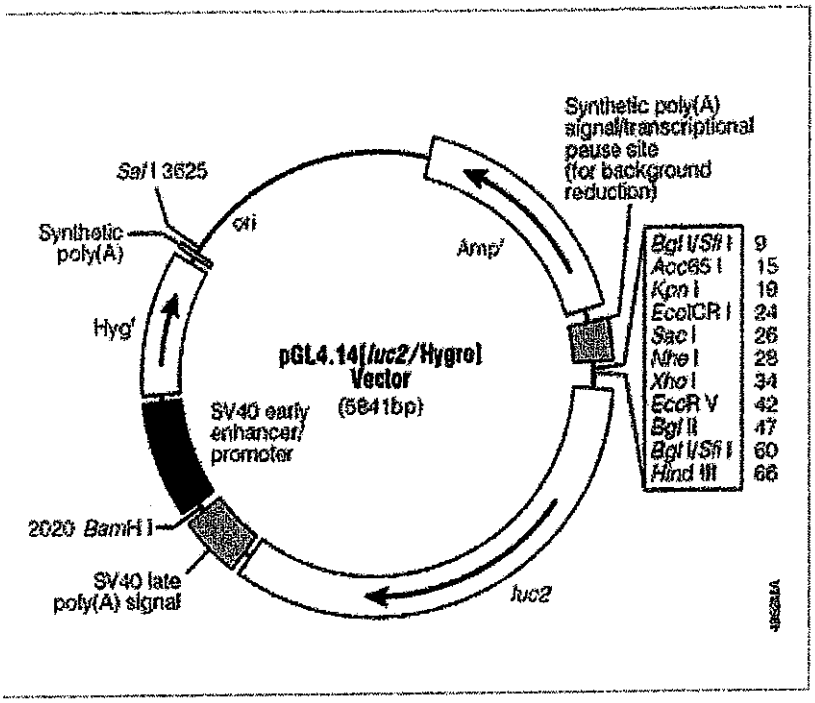

Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

LIST BIOLOGICAL LABORATORIES, INC.

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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

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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 accomplir 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

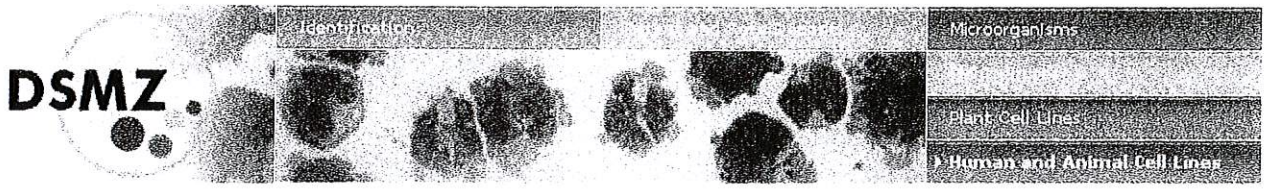
Product Description

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

Print this Page

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. [58053] Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22133]		
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

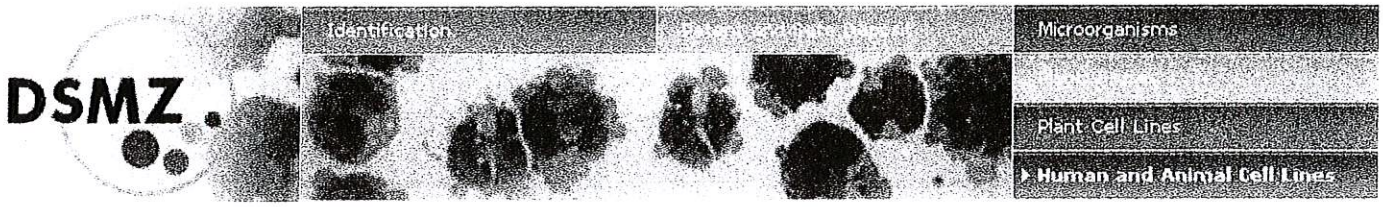
DSMZ Cell Culture Data

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 -

Back



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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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Cell Biology

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

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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...