Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

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

Mevan Perera

Yuhua Chen

Roja Rohani

Vasiliki Economopoulos

Jennifer Noad

Shruti Krishnamoorthy

Laura GonzalezLara

Carmen Simedrea

Dean Percy

Christiane Mallet

Catherine Ramsay

Emeline Ribot

Additional Personnel
(Please list additional personnel here)

Gabrielle Siegers

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not abbreviate.**
Approved Microorganisms		
Approved Primary and Established Cells	Human (established): THP-1, FaDu Luc2.11, MDA-MB-231(PA), MDA-MB-231BR. Rodent (established): B16F10, Glioma-261, Glioma-261 RFP. C4-2 Cell line, C4-2B Cell Line. PC-3/M Cell line (Human Prostate Tumour	Human(established)EM-2,EM-2eGFPluc, MEC-1,TMD2,RAJI,RAJIeGFPluc,K562, K562eGFPluc,LCLs-CS/WO. Primary Cells ~~PBMCs,GDTs,GDT clones~~ (variolous
Approved Use of Human Source Material		healthy donors),therapy mouse EM2eGFPluc.Horse Embryonic stem cell.
Approved Genetic Modifications (Plasmids/Vectors)	pCMV-DSRed Express, pGL4.14. Androgen independent cell line, clone of C4. Androgen Independent Cell line colony of C4	
Approved Use of Animals	Nu/Nu (nude) mice, C57B1/6 mice AUS#: ~~2006-013-03~~, 2007-041-03, ~~2006-008-01~~	CB17/SCID 2010-210,2009-042

Approved Biological Toxin(s)	Pertussis	

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

Signature of Permit Holder: _____

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

Date of Last Biohazardous Agents Registry Form: Dec 19, 2008

Date of Last Modification (if applicable): Feb 26, 2010

BioSafety Officer(s): _____ Sept-03/10

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

To Whom it May Concern,

Dr. Thomas Koch is collaborating researcher with Dr. Paula Foster from the University of Guelph. His lab harvest Embryonic Stem Cells from Horse (HESC). Dr. Paula Foster would like to use this primary horse stem cells line for cellular MRI research.

These cells will be cultured, labeled with paramagnetic iron oxide particles and analyzed using Perls Prussian Blue (PPB) and Fluorescent staining. In addition, various markers will be monitored using flow cytometry to analyze cell viability.

This work will be performed in Dr. Paula Foster's laboratory. The Protocol of labeling HESC is attached.

Please let me know if you have questions or comments.

Regards,

Yuha Chen

Mailing Address:
Dr. Paula Foster
Attn: Yuhua Chen
Attn: Cat Ramsay

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Protocol for labeling *HESC* with Molday ION Rhodamine B

- Plating (Day 1):
  - ✓ Remove medium
  - ✓ Wash with 5ml of PBS
  - ✓ Add 1 ml of Trypsin + EDTA
  - ✓ Incubate for 3 minutes at 37°C and 5% CO_2
  - ✓ Add 5ml of DMEM Low Glucose + 30% FBS + pen/strep to stop the reaction
  - ✓ Mix well and transfer to a 15ml tube
  - ✓ Centrifuge for 5 minutes at 800 RPM
  - ✓ Aspirate the supernatant and resuspend the cells in 5ml of DMEM + 30% FBS or 5mL of HBSS
  - ✓ Count cells
  - ✓ Plate $1.5x10^5$ cells/mL of media
  - ✓ Agitate the plate to distribute cells
  - ✓ Check under the microscope for the presence of cells
  - ✓ Incubate for 24 hours at 37°C and 5% CO_2

- Labeling with Molday ION Rhodamine B (Day 2):
  - ✓ Add 50ug/mL of USPIO to each well

- Harvesting (Day 3):
  - ✓ Aspirate old medium
  - ✓ Wash each well with 2-5ml of HBSS ***3 TIMES***
  - ✓ Add 0.5-1ml of Trypsin+ EDTA to each well
  - ✓ Incubate for 3 minutes at 37°C and 5% CO_2
  - ✓ Add 2-5ml of DMEM+10% FBS to all wells combined to stop the reaction
  - ✓ Mix well and transfer to a 15ml falcon tube
  - ✓ Centrifuge for 5 minutes at 800 RPM
  - ✓ Aspirate off the supernatant and resuspend cells in 5ml PBS
  - ✓ Repeat a total of 3 times to ensure all unincorporated label is washed away
  - ✓ Resuspend in 1-5mL of HBSS depending on experiment or pellet size
  - ✓ Count and use cells as required

- Analysis of Cells:
  - ✓ Trypan Blue analysis to check population viability after labeling
  - ✓ Cytospin 2x105 cells/300uL and stain using the Perls Prussion Blue (PPB) staining method to analyze iron content
  - ✓ Take fluorescent images to visualize particle uptake
  - ✓ Aliquot and stain with Annexin V and 7AAD to quantify cell membrane integrity /cell viability
  - ✓ Make gelatin phantoms to ensure visualization of cells using MRI technology

To Whom it May Concern,

Dr. Gabrielle Siegers is collaborating research works with Dr. Paula Foster. She would like to use the following immortalized human cell lines for cancer research:

EM-2, EM-2eGFPluc
MEC-1
TMD2
RAJI, RAJIeGFPluc
K562, K562eGFPluc
LCLs
These cells will be cultured and used as targets in *in vitro* cytotoxicity assays. In addition, various surface markers will be monitored over time using flow cytometry.

Dr. Siegers would also like to work with the following primary human cells:

Therapy mouse EM-2eGFPluc (from bone marrow)
Peripheral blood mononuclear cells (PBMCs)
Gamma delta T cells (GDTCs)
Gamma delta T cell clones
We will culture these cells and use them in *in vitro* cytotoxicity assays with the above target lines. We plan to label GDTCs with super paramagnetic iron oxide particles or gadolinium to permit their detection by magnetic resonance imaging. If labeling is successful, then the cells will be injected into the mouse model described above to monitor GDTC tracking in the context of CML minimal disease. The information for each cell line is attached.

This work will be performed in Dr. Greg Dekaban's human cell culture facility ~ Rm 2222

Please let me know if you have questions or comments.

Regards,

Yuhua chen.
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ALL CELL LINES WILL BE TREATED AS BIOSAFETY LEVEL 2.

Cell Lines

Name	Type	Origin transduced RADIL?	myc test
EM-2	Ph(+)leukemia	Dr. Keating gen	neg
EM-2eGFPluc	Ph(+) leukemia	T. Felizardo yes – ok	neg
MEC-1	B-CLL	J. Schueler, Germany	neg
TMD2	B-CLL	S. Tohda, Japan	
RAJI	Burkitt's lymphoma	UHN collaborator	neg
RAJIeGFPluc	Burkitt's lymphoma	T. Felizardo	neg
K562 Ph(+)	leukemia		neg
K562eGFPluc	Ph(+) leukemia	T. Felizardo yes - ok	neg
LCLs - CS/WO	EBV-transformed	B Siegers/Keating lab	neg
Primary Cells			
therapy mouse EM2eGFPluc			
PBMCs		various healthy donors	
GDTs		various healthy donors	
GDT clones		various healthy donors	

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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TMD2

Cell Culture [1]

Culture medium	80% α-MEM + 20% FBS + 5 ng/ml IL-3
Viral status	EBNA$^-$
Authentication	yes (by *IGH* rearrangement)
Primary reference	Tohda et al. [1]
Availability	not known (no response to request)

Clinical Data [1]

Patient	67-year-old male
Disease diagnosis	B-CLL
Treatment status	acute phase, lymphoblastic transformation
Specimen site	peripheral blood
Established	1990

Immunoprofile [1]

T-/NK cell	CD2$^-$
B-cell	CD10$^+$, CD19$^+$, CD20$^+$, sIgMκ$^+$
Myelomonocytic	CD13$^-$, CD14$^-$, CD33$^-$
Non-lineage/stem cell	HLA-DR$^+$
Adhesion	CD11b$^-$
Cytokine receptor	CD123$^+$

Genetics [1]

Karyotype	46, X, -Y, 5q-, 6p+, -8, 9p-, 11q+, 17p-, +2mar
Receptor genes	*IGH* RR, *TRB* G

Functional Features [1,2]

Colony formation	in methylcellulose
Cytochemistry	ANBE$^-$, CAE$^-$, MPO$^-$, PAS$^-$
Cytokine response	IL-3-dependent

References

[1] Tohda S et al., Blood 78: 1789-1794 (1991).
[2] Nara N, Leukemia Lymphoma 7: 331-335 (1992) (mini-review on TMD2).

- ❑ Mature EBV-negative B-cell line established from B-CLL in transformation.
- ❑ Unexpected constitutively IL-3-dependent - limited karyotypic data available.

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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Gender:	male
Ethnicity:	Black
Comments:	The Raji line of lymphoblast-like cells was established by R.J.V. Pulvertaft in 1963 from a Burkitt's lymphoma of the left maxilla of an 11-year-old Black male. [22142] EBNA positive.
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%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by addition of fresh medium or replacement of medium. Alternatively the cells may be collected by centrifugation. Cultures can then be established by resuspending the cells in fresh medium at 4 X 10(5) viable cells/ml. A maximum of 3 X 10(6) viable cells/ml is obtainable. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CCL-214

References:

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

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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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Product Information Sheet

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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Subculturing:	**Protocol:** Cultures can be maintained by the addition or replacement of fresh medium. Start new cultures at 1 X 10 exp5 viable cells/ml. Subculture at 1 X 10 exp6 cells/ml. **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2005 recommended serum:ATCC 30-2020 purified DNA:ATCC CCL-243D purified RNA:ATCC CCL-243R
References:	867: Koeffler HP, Golde DW. Human myeloid leukemia cell lines: a review. Blood 56: 344-350, 1980. PubMed: 6996765 1101: Ortaldo JR, et al. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. J. Natl. Cancer Inst. 59: 77-82, 1977. PubMed: 69036 22609: Lozzio CB, Lozzio BB. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45: 321-334, 1975. PubMed: 163658 26059: Lozzio BB, Lozzio CB. Properties and usefulness of the original K-562 human myelogenous leukemia cell line. Leuk. Res. 3: 363-370, 1979. PubMed: 95026 26060: Andersson LC, et al. K562--a human erythroleukemic cell line. Int. J. Cancer 23: 143-147, 1979. PubMed: 367973 26061: Lozzio BB, et al. A multipotential leukemia cell line (K-562) of human origin. Proc. Soc. Exp. Biol. Med. 166: 546-550, 1981. PubMed: 7194480 26063: Dimery IW, et al. Variation amongst K562 cell cultures. Exp. Hematol. 11: 601-610, 1983. PubMed: 6576909 32357: Chan YJ, et al. Two distinct upstream regulatory domains containing multicopy cellular transcription factor binding sites provide basal repression and inducible enhancer characteristics to the immediate-early IES (US3) promoter from human cytomegalovirus. J. Virol. 70: 5312-5328, 1996. PubMed: 8764042 32396: Kolanus W, et al. alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule. Cell 86: 233-242, 1996. PubMed: 8706128 32446: Gan W, Rhoads RE. Internal initiation of translation directed by the 5'-untranslated region of the mRNA for eIF4G, a factor involved in the picornavirus-induced switch from cap-dependent to internal initiation. J. Biol. Chem. 271: 623-626, 1996. PubMed: 8557663 32561: Tiffany HL, et al. Enhanced expression of the eosinophil-derived neurotoin ribonuclease (RNS2) gene

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48833: Pross HF, et al. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J. Clin. Immunol. 1: 51-63, 1981. PubMed: 7334070
61237: Chen TR. Modal karyotype of human leukemia cell line, K562 (ATCCCCL 243). Cancer Genet. Cytogenet. 17: 55-60, 1985. PubMed: 3857109
61327: Wu SQ, et al. Extensive amplification of bcr/abl fusion genes clustered on three marker chromosomes in human leukemic cell line K-56. Leukemia 9: 858-862, 1995. PubMed: 7769849

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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.

PBMC isolation from human blood (~50 ml)

Blood is collected from healthy donors in heparin tubes. Slowly invert 8 x to mix, then transfer ~18 ml/tube to 50 ml Falcon tubes. Add 22 ml PBS. Add 10 ml Ficoll to 6- 50 ml Falcon tubes. Overlay carefully with 20 ml blood/PBS using a Pasteur pipette. Centrifuge at 800g for 30 min without brake, using acceleration and deceleration settings of 5 and 1 respectively. Remove serum (upper layer) and harvest cloudy lymphocyte layer using Pasteur pipette. Transfer lymphocytes into 2- 50 ml Falcon tubes. Add PBS to 45 ml and centrifuge 10 min at 1800 rpm using acceleration and deceleration settings of 5 and 4, respectively. Re-suspend pellets in 10 ml MACS buffer (PBS, 2mM EDTA, 0.5% BSA or human serum) and centrifuge 5 min, 1500 rpm, same settings. Re-suspend pellet in 1 ml MACS buffer and count in 3% Acetic Acid in Methanol blue at 1:250 dilution. Proceed with MACS separation protocol.

γδ T cell isolation and expansion protocol
GS, Dec 2009

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.

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.

Research

University Health Network

September 20, 2007

UHN Research Safety Training

Certificate of Completion

Presented to

Gabrielle Siegers

Jacquie Tumak

Wiebke K Wilkens

WILKENS Health & Safety Solutions

Jacquie Tumak Wiebke Wilkens

Occupational Health and Safety Training Registration Update Confirmation

Transaction Record:

The following reflects the changes made to your Health and Safety training sessions.

This is the only confirmation you will receive for your enrollment. **PLEASE PRINT FOR YOUR RECORDS** .

UWO ID:	020060014
First Name	Gabrielle
Last Name	Siegers
UWO Email:	gsiegers@imaging.robarts.ca
Department:	Other
Work Phone:	ext 24316
Campus Address:	Robarts Research 1200
Leader / Manager:	Paula Foster

Session Enrolment

New Employee Health and Safety Orientation
Support Services Building - Rm 4210
Sept 20, 2010 11:00 a.m. - 12:00 p.m.

Laboratory and Environmental/Waste Management Safety
Support Services Building - Rm 4210
Sept 13, 2010 9:00 a.m. - 12:00 p.m.

Biosafety
Medical Sciences - Rm 384
Sept 15, 2010 1:30 p.m. - 4:30 p.m.

Radiation Safety
Support Services Building - Room 4220
Sept 16, 2010 9:00 a.m. - 4:00 p.m.

PLEASE ARRIVE 10 MINUTES BEFORE SESSION START TIME.

- Update Enrolment Request
- Back to Human Resource Services

Please contact us if you experience any problems using this application.

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

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

Mevan Perera

Vasiliki Economopoulos

Jennifer Noad

Shruti Krishnamoorthy

Yuhua Chen

Roja Rohani

Laura GonzalezLara

~~Jonathon Snir~~

Christiane Mallet

Catherine Ramsay

Emeline Ribot

Additional Personnel
(Please list additional personnel here)

Dean Percy

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. ***
Approved Microorganisms		
Approved Cells	Human (established): THP-1, FaDu Luc2.11, MDA-MB-231PA, MDA-MB-231BR. Rodent (established): B16F10, Glioma-261, Glioma-261 RFP. C4-2 Cell line, C4-2B Cell Line. PC-3/M Cell line (Human Prostate Tumour Lines)	①MDA-MB-231-Luc-D3H2LN (Human ②KHYG-1 (Human) ③MDA-MB-435 (Human)
Approved Use of Human Source Material		Level 1 cell lines only.
Approved GMO	pCMV-DSRed Express, pGL4.14. Androgen independent cell line, clone of C4. Androgen Independent Cell line colony of C4	
Approved use of Animals	Nu/Nu (nude) mice, C57B1/6 mice AUS#: 2006-013-03, 2007-041-03, 2006-008-01	

Approved Toxin(s)

Pertussis	

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

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Dec 19, 2008

Date of Last Modification (if applicable): Jul 17, 2009

BioSafety Officer(s): JStanley Feb 26/10 _____ Feb.09/10

Chair, Biohazards Subcommittee: _____

NB. Perform injections on anaesthetized rodents. JJ.

To Whom it May Concern,

Dr. Paula Foster would like to use MDA-MB-231-Iuc-D3H2LN, MDA-MB-435 and KHYG-1 cells line for cancer research. The cells will be cultured and labeled with super paramagnetic iron oxide particles or gadolinium to permit their detection by magnetic resonance imaging. If the cell labeling is effective then the cells will be injected/implanted into mice for cancer imaging project. The information of each cell line is enclosed.

Please let me know if you have questions or comments.

Regards,

Yuhua chen.

Mailing Address:
Dr. Paula Foster
Attn: Yuhua Chen
Rm# 3296
P.O. Box 5015
100 Perth Dr
London ,ON
N6A 5K8
Phon# 519-663-5777 Ext: 24133
Fax# 519-931-5224
Email: Ychen@robarts.ca

MDA-MB-231-luc-D3H2LN
Cell Line Information Sheet

General Information:

Designation:	MDA-MB-231-luc-D3H2LN
Tissue:	Human: adenocarcinoma; mammary gland; pleural effusion
Source of Parental Line:	ATCC (#HTB-26)
Derivation:	Harvested from metastatic lymph node tissue resulting spontaneously from an orthotopic MB-231-luc-D3H1 tumor after 12 weeks of in vivo growth.
Pathogen Testing:	Mouse Pathogen Test: IMPACT Profile I (PCR) at the University of Missouri Research Animal Diagnostic and Investigative Laboratory MDA-MB-231 Parental (passage 4): Negative 1/24/00 MDA-MB-231-luc-D3H2LN (passage 8): Negative 2/8/2008

Cotransfection:

Plasmids:	1) pGL3 control red (SV40-luc) (originally from Promega, construct from Chris Contag Lab at Stanford) 2) pSV40/Zeo (Invitrogen)
Transfection Method:	Lipofectamine/Plus Reagent (Invitrogen)

In Vitro Growth:

Recommended Media:	Eagle's MEM (500 ml) ATCC Cat. No.30-2003 Supplement the above with: Final concentration 50 ml FBS (Hyclone) 10%
Cell Doubling Time:	Approximately 24 hours (similar to parental cell line)
Split Ratio:	1:5 to 1:10, every 2-3 days using 0.25% Trypsin with EDTA
Zeocin Sensitivity:	50-100 µg/ml (If culturing cells >2 months, add 75ug/ml Zeocin in growth media to maintain optimum bioluminescence)

In Vitro Bioluminescence:

Lower Limit of Detection:	Able to detect ~20 cells in 200µl using IVIS® system when imaged at 1 min, 10 bin, level B/FOV 15 Bioluminescence: 189-208 photons/second/cell
Stability Over Time:	Signals stable for at least 2 months in culture without Zeocin selection; Signal drops by ~40% after 2 months

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:	**HTB-26™** Order this Item	**Price:**	**$244.00**
Designations:	MDA-MB-231	**Depositors:**	R Cailleau
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Preparation	**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

Berkeley Lab

Home
Breast Cancer Cell Lines
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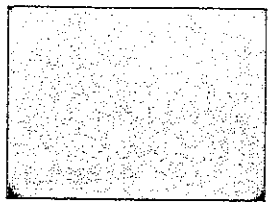
Resources

Contact
Lawrence Berkeley
National Laboratory
717 Potter Street
Berkeley, CA 94710-2722

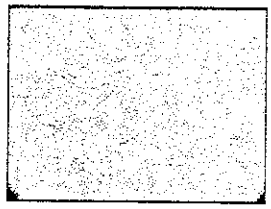
« MDAMB415 MDAMB436 »

MDAMB435

ATCC	
Specimen Origin	
Source	Georgetown
Organism	Homo Sapiens (Human)
Tissue	Skin
Tumourigenic	
Morphology	Epithelial
Age Status	
Ethnicity	
Gender	Female
Cytogenetic Analysis	
Growth Properties	Adherent
Doubling Time	
Culture Media	DMEM, 10%FBS
Split Ratio	
Freeze Media	culture medium 95%; DMSO, 5%
Comments	There is debate regarding whether this is a breast cancer cell lines or not, since it clustered with melanoma samples in PMID: 12354931, 15679052, 15150101. A recent article by Rae et al (PMID 17004106) definitively identifies this as a line derived from the M14 melanoma cell line. For more information see: http://dtp.nci.nih.gov/docs/misc/common_files/mda-mb-435-update.html
References	
Mutations	



10X



20X

Synonymous Cell Lines

MDAMB435S

MDA-MB-435, a member of the NCI-DTP panel of 60 human tumor cell lines, has been used for decades as a model of metastatic human breast cancer. This cell line was derived at M.D. Anderson in 1976 from a pleural effusion from a 31-year old woman with a history of breast cancer (Cailleau R, Olive M, Cruciger QV. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro. 1978 Nov;14(11):911-5. ; Brinkley BR, Beall PT, Wible LJ, Mace ML, Turner DS, Cailleau RM. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 1980 Sep;40(9):3118-29.) Further background information on this cell line may be found at the M.D. Anderson Breast Cancer Cell Line Database.

Recent advances in gene expression analysis allow the opportunity to more fully characterize tumor cell lines. Analysis of MDA-MB-435, in conjunction with the rest of the NCI60 panel, revealed that the pattern of gene expression for MDA-MB-435 more closely resembled that of melanoma cell lines than of other breast tumor lines (Ross et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat Genet 2000 Mar;24(3):227-3.)

These findings prompted Ellison et al. to undertake a more detailed study of the characteristics of MDA-MB-435 (Ellison G, Klinowska T, Westwood RF, Docter E, French T, Fox JC. Further evidence to support the melanocytic origin of MDA-MB-435. Mol Pathol. 2002 Oct;55(5):294-9.). They measured expression of several breast-specific genes and several melanoma-specific genes in MDA-MB-435 (obtained from the American Type Culture Collection), as well as in other breast tumor cell lines, melanoma cell lines and normal breast. Breast-specific genes were not detectably expressed in MDA-MB-435 or in the melanoma lines, but were detected in most of the breast tumor cell lines as well as normal breast. However, melanocyte-specific genes were expressed in MDA-MB-435, as well as in most of the melanoma lines, but were not detectable in the other breast tumor cell lines. Additionally, xenografts of MDA-MB-435 implanted into mammary fat pads of female SCID mice showed immunohistochemical staining consistent with melanocytic origin.

More recently single nucleotide polymorphism (SNP) array analysis revealed that MDA-MB-435 is derived from the same individual as the melanoma cell line M14 (Garraway LA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005 Jul 7;436(7047):117-22. ; http://www.sanger.ac.uk/genetics/CGP/NCI60/).

The NCI Developmental Therapeutics Program obtained MDA-MB-435 from Dr. Patricia Steeg (NCI) -- Dr. Steeg obtained the line from M.D. Anderson. The DTP has obtained DNA fingerprinting analysis of the MDA-MB-435 in the DTP repository, as well as MDA-MB-435 from the ATCC (which obtained their sample from M.D. Anderson). DNA fingerprinting on all MDA-MB-435 samples are consistent with their derivation from the same individual. Thus the mix-up with the melanoma cell line M14 likely happened early in the history of the cell line.

Note added 8/5/2009: The panel designation for this cell line continues to be a topic for discussion, as seen in a recent publication by Chambers.
(MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 2009 Jul 1;69(13):5292-3.)

Note added 8/2007: A recent publication by Rae et al. used karyotype, CGH, and microsatalite polymorphism analyses, combined with bioinformatics analysis of gene expression and SNP data and concluded that "All currently available stocks of MDA-MB-435 cells are derived from the M14 melanoma cell line". (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. 2007 Jul;104(1):13-9.)

Health Science Research Resources Bank
Japan Health Sciences Foundation

http://www.jhsf.or.jp/cgi-bin/HSRRB/C_ViewDetail.cgi?jcrb=JCRB0156

JCRB No.	JCRB0156
Cell Name	KHYG-1
Profile	Human natural killer cell line with a p53 point mutation as a model for p53-associated leukemogenesis and a model for differentiation of NK/T cells.
Animal	human
Species	Homo sapiens
Sex	F
Age	45-year-old
Tissue	peripheral blood
Case History	aggressive NK cell leukemia
Metastasis	
Genetics	p53 point mutation and cytogenetic characteristics.
Lifespan	infinite
Morphology	lymphocyte-like
Characteristics	high NK/LAK activity, IFN gamma production etc.
Classification	tumor
Establisher	Yagita,M.
Depositor	Yagita,M.
Medium	RPMI1640 medium with 10% fetal calf serum and 100 units/ml of rIL-2(Shionogi).
Passage Method	Simple dilution because of suspension cell culture. Subculture every 3-4 days with split ratio = 1/4.
Passage Cell No.	Split 1/4 every 3-4 days.

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

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

Mevan Perera

Vasiliki Economopoulos

Jennifer Noad

Shruti Krishnamoorthy

Yuhua Chen

Roja Rohani

Laura GonzalezLara

Jonathon Snir

Christiane Mallet

Catherine Ramsay

Emeline Ribot

Additional Personnel
(Please list additional personnel here)

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Dec 19, 2008

Signature of Permit Holder: Paula Foster

BioSafety Officer(s): J Turley July 17/09

Chair,Biohazards Subcommittee: E M Kidder

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		PC-3/M Cell line
Approved Cells	Human (established): THP-1, FaDu Luc2.11, MDA-MB-231PA, MDA-MB-231BR. Rodent (established): B16F10, Glioma-261, Glioma-261 RFP. C4-2 Cell line, C4-2B Cell Line	
Approved Use of Human Source Material		
Approved GMO	pCMV-DSRed Express, pGL4.14. Androgen independent cell line, clone of C4. Androgen Independent Cell line colony of C4	Human prostate tumor lines
Approved use of Animals	Nu/Nu (nude) mice, C57B1/6 mice AUS#: 2006-013-03, 2007-041-03, 2006-008-01	
Approved Toxin(s)	Pertussis	

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Dec 19, 2008

Signature of Permit Holder: Paula Foster

BioSafety Officer(s): JHunley July 17/09

Chair,Biohazards Subcommittee: G.M. Kidder

Subject: Re: [Fwd: Re: [Fwd: Re: Containment Level request for C4-2B cell line]]
From: pfoster@imaging.robarts.ca
Date: Mon, 13 Jul 2009 20:02:49 -0400 (EDT)
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer

the cell line C4-2B does not contain human pathogens and has not been transformed with human pathogens.

-Paula

→ level 1 cell line per July Biohazards Subcommittee meeting ok.

-------- Original Message --------
Subject: Re: [Fwd: Re: Containment Level request for C4-2B cell line]
Date: Mon, 13 Jul 2009 15:48:46 -0400 (EDT)
From: pgareau@imaging.robarts.ca
To: Jennifer Stanley <jstanle2@uwo.ca>
References: <4A5B7F7E.7010903@uwo.ca>

Paula's new email is pfoster@imaging.robarts.ca

> Hi Paula
> Can you confirm that this cell line does not contain human pathogens and has not been transformed with human pathogens (see note below from PHAC - the Public Health Agency of Canada).
> Jennifer
>
> -------- Original Message --------
> Subject: Re: Containment Level request for C4-2B cell line
> Date: Mon, 13 Jul 2009 13:35:58 -0400
> From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
> To: Jennifer Stanley <jstanle2@uwo.ca>
>
> Hi Jennifer,
>
> If the cell line does not contain human pathogens (i.e. EBV) or was not transformed with a human pathogens (i.e. SV40) then as far as it concerns
> our office, it does not require a containment level 2 or more laboratory.
> Also, you can refer to our LBGs, we have a general section on risk assessment and a more specific one on cell line more in our Laboratory Biosafety Guidelines (Chapter 2 and Chapter 7.3).
>
> Hope this helps.
>
> Genevieve Lacroix, M.Sc.
> A/Head, Importation and Biosafety Programs
> Chef intérimaire/Importation et servuce de biosécurité
> Office of Laboratory Security / Bureau de la sécurité des laboratoires
> Public Health Agency of Canada / Agence de la santé publique du Canada
> 100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
> Tel: (613) 946-6982
> Fax: (613)941-0596
> genevieve_lacroix@phac-aspc.gc.ca
> http://www.phac-aspc.gc.ca/ols-bsl/index.html

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

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

Mevan Perera

Vasiliki Economopoulos

Jennifer Noad

Shruti Krishnamoorthy

Yuhua Chen

Roja Rohani

Laura GonzalezLara

Jonathon Snir

Christiane Mallet

Catherine Ramsay

Emeline Ribot

Additional Personnel
(Please list additional personnel here)

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Dec 19, 2008

Signature of Permit Holder: Paula Foster

BioSafety Officer(s): [illegible] May 15/09

Chair,Biohazards Subcommittee: [illegible]

Modification Form for Permit BIO-RRI-0032

Permit Holder: Paula Foster

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		C4-2B Cell Line
Approved Cells	Human (established): THP-1, FaDu Luc2.11, MDA-MB-231PA, MDA-MB-231BR. Rodent (established): B16F10, Glioma-261, Glioma-261 RFP. C4-2 Cell line.	
Approved Use of Human Source Material		
Approved GMO	pCMV-DSRed Express, pGL4.14. Androgen independent cell line, clone of C4	Androgen independent Cell line - Colone of C4.
Approved use of Animals	Nu/Nu (nude) mice, C57B1/6 mice AUS#: 2006-013-03, 2007-041-03, 2006-008-01	
Approved Toxin(s)	Pertussis	

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Dec 19, 2008

Signature of Permit Holder: Paula Foster

BioSafety Officer(s): [illegible] May 15/09

Chair, Biohazards Subcommittee: G.R. Kelder

To Whom it May Concern,

Dr. Foster, of the Robarts Research Institute at The University of Western Ontario, would like to purchase the C4-2B cell line. The cells will be labeled, by co-culture of transfection, with super paramagnetic iron oxide particles to permit their detection by magnetic resonance imaging. If the cell labeling is effective then the cells will be it is injected/implanted into mice for a cancer imaging project.

Mailing Address:
Dr. Paula Foster
Attn: Yuhua Chen/Cat Ramsay
P.O. Box 5015
100 Perth Dr
London ,ON
N6A 5K8

Proposed use of C4-2B Cells

These cells will be used to investigate a mouse model of prostate cancer in which cell and tumour progression will be monitored using magnetic resonance imaging. We will culture and label the cells with a contrast agent containing iron or gadolinium, in our tissue culture room which is approved for level 2 biohazards. Then, the cells will be injected into immune-compromised mice, which will be housed in a barrier facility at the University of Western Ontario. The mice will be imaged periodically with MRI at the Robarts Research Institute.

ViroMed Laboratories
A LabCorp Company

CHuvá cells

Human Prostatic Carcinoma Cell Lines

Catalog Number	Cell Line Abbreviation	Product Description	Price
12-100	LNCaP	Direct parental prostate cancer cell line	$ 210.00
12-101	C4	Androgen dependent cell line, clone of LNCap	367.50
12-102	C4-2	Androgen independent cell line, clone of C4	367.50
12-103	C4-2B	Androgen independent cell line, clone of C4, developed in bone	367.50

A materials transfer agreement (MTA) is required prior to obtaining these cell lines.

Shipping Cost: Please contact your ViroMed account manager for specific information regarding domestic and international shipping costs.

For further information, please contact ViroMed Client Services at 800-582-0077.

Service ordering procedure to obtain

www.viromed.com

Revised February 19, 2009

ViroMed Laboratories
6101 Blue Circle Drive
Minnetonka, MN 55343-9108
Telephone: 800-582-0077
www.ViroMed.com

Chung Cancer Cell Lines – Procedure to Obtain

Submit the following information via email:

1. Chief Investigator's Name
2. Intended usage statement
3. Exact shipping address (and billing, if different; we also accept Visa/MC for payment)

This information will then be forwarded and,

4. A Materials Transfer Agreement (MTA) will then be sent to you via email by our Sales Administration Dept. The above 3 items will be added to the MTA.

PLEASE NOTE: The terms of the MTA cannot be altered or changed. We can only accept the MTA as originally prepared. If terms of the MTA are altered, we will submit the changes to both our legal dept and MD Anderson Cancer Center for their approval. The approval process can take several weeks.

5. When the signed MTA (2 original copies) is received back, the ordering process can then proceed with verification of order and payment information.

For International shipments: Credit card or direct bank transfer is the preferred payment.

6. The order is then submitted to our production dept. We normally ship these cells out on a Monday or Tuesday; the orders may take up to several weeks to ship.

Reference Publications

1. LNCaP Human Prostrate Cancer Progression Model. Leland W.K. Chung, Ph.D. Urol Oncol 1996; 2:000-000
2. Androgen and retinoic acid interaction in LNCaP cells, effects on cell proliferation and expression of retinoic acid receptors and epidermal growth factor receptor. Ming-tang Li, Frank Richter, Chawnshang Chang, Robert J Irwin, Hosea FS Huang. BMC Cancer 2002 2:16.
3. Androgen-independent Cancer Progression and Bone Metastasis in the LNCaP Model of Human Prostrate Cancer. Cancer Research 54, 2577-2581, May 15, 1994.
4. Derivation of Androgen-Independent Human LNCaP Prostatic Cancer Cell Sublines: Role of Bone Stromal Cells. Int.J.Cancer 57, 406-412, 1994.

CELL-LINE SPECIFICATIONS

Name of Cell Line: C4-2

Origin: The human prostatic carcinoma cell line, *LNCaP* (1×10^6 cells; passage # 29) previously described [1] was co-inoculated into an athymic male nude mouse with (1×10^6) human fibroblasts derived from an osteosarcoma (cell line MS). The nude mouse host was castrated after 8 weeks incubation. A tumor specimen was excised after a total of 12 weeks. The *C4* cell line constitutes the *in vitro* cultured subline grown from the murine host's tumor [2]. When the *C4* sub-line was subsequently co-inoculated with MS osteosarcoma fibroblasts in a castrated athymic male nude mouse host for another 12 weeks by the same protocol described above. Prostatic epithelial cells cultured from the resultant tumor in this host constituted the *C4-2* subline[3].

Cell Line characteristics:
Morphology: fibroblast-like growth in tissue culture flasks; may form concentric zones of low cell growth upon reaching confluence.

Tumorigenicity & Osseous Metastasis: Orthotopic administration of 1×10^6 resuspended C4-2 cells in both intact and castrated athymic male nude mice yielded 100% tumorigenicity (20/20 and 14/14, respectively). Osseous prostate cancer metasteses were detected in both intact and castrated murine hosts (2/20 and 3/14, respectively) [3].

Soft-agar cloning: When 1×10^4 resuspended C4-2 cells were cloned on 0.3% soft agarose supplemented with 5% FBS and 2% TCM, colony formation was scored as 313 ± 15.3 relative to 12 ± 2.5 from the parental LNCaP cell line [2].

Androgen sensitivity: LNCaP sublines are androgen-independent.

Maintenance of C4-2, C4 and parental LNCaP cell lines:

Freezing Media: 10% DMSO + 90% T Media (containing 20% FBS)

Freezing Procedure: Freeze cells when cell layer becomes 90% confluent. Do not freeze at a confluency higher than 90%. Change media 1 day prior to freezing cells. Freeze at 1.5×10^6 cells per vial.

Frequency of Passage: Passage should be performed when confluency reaches 80-90%. If culture is allowed to remain too high of a confluency for more than a day or two, cells will begin to slough away from the monolayer. Over-grown passages can be difficult to re-plate as single cells. If cell number is too low when passing, cell growth tends to aggregate in isolated areas instead of growing and spreading across the flask.

Passing procedure: Cells are washed in PBS, pH 7.2. These cells are passed using a 0.05% trypsin/0.53mM EDTA solution. Apply trypsin/EDTA in the following volumes:

1.5 – 2ml/T25 flask
4 – 5 ml/T75 flask
5 – 6 ml/T175 flask

Leave trypsin/EDTA solution on cells for about 5 minutes, checking them frequently. Neutralize trypsinized cells with T media containing 5% FBS. Always centrifuge the trypsin/EDTA out of the culture and transfer cells to a new flask. Media volumes are as follows:

5ml media/T25 flask
15ml media/T75 flask
30ml media/175T flask

[1] Horoszewicz, JS et al. *Cancer Research* 43:1809-1818, 1983.

[2] Wu et al. Derivation of androgen-independent human LNCaP prostatic cell sublines: Role of bone stromal cells. *International Journal of Cancer* 57:406-412, 1994.

[3] Thalmann et al. Androgen-independent cancer progression and bone metastatis in the LNCaP model of human prostate cancer. *Cancer Research* 54:2577-2581, 1994.

CELL LINE THAWING & REVITALIZATION PROTOCOL

(It is recommended that the "C-4" series of LNCaP-derived cell lines be stored long-term in liquid nitrogen.)

1) Remove cell storage vial from liquid nitrogen. Thaw rapidly in a 37° C. water bath.
2) Immediately transfer thawed cell solution to a 15 ml centrifuge tube.
3) Slowly resuspend thawed cells in 10 ml of growth media.
4) Centrifuge at 4°C or room temperature for 7-8 minutes at 800 rpm.
5) Aspirate media from cell pellet and resuspend in 15 ml fresh media.
6) Transfer into T-75 tissue culture flask and place in 37°C (5% CO2) incubator.
7) Do NOT perform media exchange the day after thaw date. Perform media exchange only after cells have sufficiently attached to surface of flask. Because these cells may take 1-3 days to adhere to the flask surface, premature media exchange could result in the loss of unattached viable cells.

The LNCaP cell line may require 2-3 days for a sufficient amount of cells to attach to the surface of the flask.

The C4, C4-2 and C4-2Bone cell lines typically will adhere in less 2 days.

PRODUCT AND MEDIA INFORMATION FOR LNCAP, C4, C4-2 AND C4-2 BONE

T-Media:

DMEM	liquid with sodium bicarbonate	Vendor: Cat #: Package size:	Fisher BW12-741F 500 ml
HAM'S F12	liquid with sodium bicarbonate	Vendor: Cat #: Package size:	Fisher BW12-615F 500 ml
FBS	Premium Heat inactivated	Vendor: Cat #: Package size:	Fisher BW14-503F 500 ml

T MEDIA SUPPLEMENTS: STORE at -20°C

1. Insulin

Aliquot concentration at 500x: 2.54 mg/ml in 0.1% BSA/PBS
Final concentration in 500 ml: 5 ug/ml

Vendor: Sigma
Cat #: I-1882
Package size: 100 mg

2. T3 (TRIIODO-THYRONINE)

Aliquote concentration at 500x: 6.825 ng/ml in 0.1% BSA/PBS
Final concentration in 500 ml: 13.65pg/ml

Vendor: Sigma
Cat #: T-2877
Package size: 100 mg

3. apo-TRANSFERRIN Human

Aliquot concentration at 500x: 2.2 mg/ml
Final concentration in 500 ml: 4.4 ug/ml

Vendor: Sigma
Cat #: T-4382
Package size: 1 g

4. d-BIOTIN

Aliquote concentration at 500x: 0.122 mg/ml in 0.1% BSA/PBS

Vendor: Sigma
Package size: 1 g

Final concentration in 500 ml: 0.244 ug/ml

5. ADENIN

Vendor: Sigma
Cat #: A-3159
Package size: 5 g

Aliquot concentration at 500x: 6.25 mg/ml in 0.1% BSA/PBS
Final concentration in 500 ml: 12.5 ug/ml

6. Penicillin and Streptomycin

Vendor: Mediatech
Cat #: 30-002-CI
Package size: 100 ml

Concentration:
Pen 50,000 units/ml
Strep 10,000 g/ml

Final Concentration:
Pen 100 units/ml
Strep 100 g/ml

7. TRYPSIN-EDTA (10X)
Ready to use

Vendor: Fisher
Cat #: BW17-161E
Package size: 100ml

Media Preparation:
400 ml DMEM
100 ml Ham's F12
50 ml Heat inactivated FBS (final conc. 10%)
5 ml 10X Pen/Strep (final conc. 1%)
1 ml of above supplements

Mix well and use, media will be good for 30 days.

Materials for trypsinizing the cells:
10X Trypsin (Fisher Cat # BW17-161E)
1X PBS

Procedure:

1. Thaw frozen cells quickly at 37° water bath and transfer into 15 ml tissue culture tube with 9 ml media.
2. Centrifuge at 12 RPM for 5 minutes at room temp or 4°
3. Aspirate media and wash cells with 1X PBS and centrifuge same as before.
4. Aspirate PBS and resuspend in 2 ml media and transfer to tissue culture plate or flask. Incubate at 37° with 5% CO2. **Do not disturb cells for at least four days. These cells are very sensitive and they may not adhere if they are disturbed.**

Splitting cells:

Warm up all reagents to 37° before use.

1. Split cells 1:4 when they are about 75-85% confluent by trypsinizing them with 21 ml 1x trypsin-EDTA for 5 min. at most at 37° (1ml for 100 mm plates and 25 cm2 tissue culture flask, 2 ml for 75 cm^2 flask and 3.5 ml for 150 cm^2 flask).
2. Stop trypsin with 9 ml of media; transfer cells into tissue culture tube and spin for 5 min at 1200 RPM at Room temp.
3. Aspirate media and wash cells with 10 ml 1X PBS, Centrifuge same as before and aspirate PBS.
4. Resuspend the pellet in 10 ml media and transfer into T75 tissue culture flask and incubate at 37° with 5% CO2.

Do not disturb the flask for at least two days.

MAINTENANCE OF C4, C4-2, C4-2B AND LNCaP CELL LINES:

Freezing Media: 10% DMSO + 90% T Media (containing 20% FBS)

Freezing Procedure: Freeze cells when layer becomes 90% confluent. Do not freeze at confluency higher than 90%. Change media 1 day prior to freezing cells. Freeze at 1-1.5x10^6 cells per vial.

Frequency of Passage: Passage should be performed when confluency reaches 80-90%. If culture is allowed to remain at too high of a confluency for more than a day or two, cells will begin to slough away from the monolayer. Over-grown passages can be difficult to re-plate as single cells. If cell number is too low when passing, cell growth tends to aggregate in isolated areas instead of growing and spreading across the flask.

Passing procedure: Cells are washed in PBS, pH 7.2. These cells are passed using a 0.05% trypsin/0,53mM EDTA solution. Apply trypsin/EDTA in the following volumes:

1.5 ml / T25 flask
4.0 ml / T 75 flask
5-6 ml / T 175 flask

Leave trypsin/EDTA solution on cells for about 5 minutes, checking them frequently. Neutralize trypsinized cells with T media containing 5% FBS. Always centrifuge the trypsin/EDTA out of the culture and transfer cells to a new flask. The lowest dependable amount of cells that will yield an even spread is 30,000 cells/ml – this number will vary depending each technician's own ability. Media volumes are as follows:

5 ml / T25 flask
15 ml / T75 flask
30 ml/T175 flask

Source: Cheryl Savola, PhD, ViroMed/LabCorp 10/14/04 – this document is the original information provided from UroCor/Dianon to ViroMed .

Proposed use of PC-3M Cells

These cells will be used to investigate a mouse model of prostate cancer in which cell and tumour progression will be monitored using magnetic resonance imaging. We will culture and label the cells with a contrast agent containing iron or gadolinium, in our tissue culture room which is approved for level 2 biohazards. Then, the cells will be injected into immune-compromised mice, which will be housed in a barrier facility at the University of Western Ontario. The mice will be imaged periodically with MRI at the Robarts Research Institute.

NCI-FREDERICK CANCER
DCT TUMOR REPOSITORY

AVAILABLE INFORMATION ON TRANSPLANTABLE TUMORS OR CELL LINES

DESIGNATION:	**PC-3/M**
VIAL DESIGNATION:	0502392
SPECIES:	HUMAN
TUMOR ORIGIN:	PROSTATE
HISTOLOGIC TYPE:	CARCINOMA

CELL CULTURE INFORMATION:

GROWTH MEDIUM:	RPMI 1640 + 10%FBS + 2 mM L-Glutamine
MODE OF GROWTH:	Adherent
METHOD OF HARVEST:	Trypsin/EDTA
SPLIT RATIO:	1:20 Weekly
DOUBLING TIME:	60 Hours

TUMOR PROFILE INFORMATION:

HOST STRAIN:	ATHYMIC NUDE
TISSUE IMPLANTED:	N/A
ROUTE:	N/A
SUSPENDING MEDIUM:	N/A
OPTIMAL DAY:	N/A
HOST MEDIAN SURVIVAL TIME:	N/A
METASTATIC CHARACTERISTICS:	N/A
HISTOPATHOLOGY:	N/A

SOURCE:	Dr. Edward Kaighn
REFERENCE:	Ohnuki, Y., Marnell, M., Babcock, M., Lechner, J., Kaighn, E.; Chromosomal Analysis of Human Prostatic Adenocarcinoma Cell Lines; Cancer Research 40: 524-534; March 1980.
CRYOPRESERVED:	10/27/87
VIRAL PROFILE:	NEGATIVE
OTHER INFORMATION:	The subline, PC-3/M, had a similar karyotype and retained the parental PC-3 markers. PC-3/M had a more restricted chromosomal frequency distribution range. Nearly 73% of the PC-3/M cells examined had 60 or 61 chromosomes in contrast to the wide distribution seen in PC-3. Silver staining for nucleolus organizer regions indicated that the number of functional nucleolus organizer regions in PC-3 was proportional to the number of acrocentric chromosomes.

CARE AND USE OF TUMOR LINES

The transplantable tumors are distributed as frozen vials of tumor tissues or cell suspension. Transplantable tumors as well as cell culture lines are shipped as frozen vials of tissues on dry ice. Each tumor shipment includes an information sheet showing, among other items, the proper tumor designation; Cryopreserved date, In vivo host, etc.

Requested tumors are shipped in two to three weeks after the receipt of all completed paperwork. Shipments leave the Repository no later than Wednesday in order to reach their destination on weekdays. Before the shipment leaves the Repository, the Recipient is notified by email or fax of the waybill number and carrier, to allow time for the recipient to notify the Repository or make arrangements for receipt of cells, in the event that they will not be available to receive the shipment. An invoice for payment will follow and payment is due upon receipt. When vials are received they should be cultured right away, expanded and frozen down.

SUBMISSION OF TUMORS FOR CRYOPRESERVATION

Investigators who have unique and novel experimental tumor lines and are desirous of submitting their tumors to the Repository for cryopreservation and storage should write a letter of intent to the Project Officer. Upon acceptance, the Project Officer will inform the investigator in writing, and instructions on the procedure of shipment of the tumor materials to the Repository will be given. Tumor tissues or cells (frozen or ambient) are preferred over tumor-bearing animals.

At the Repository, the tumor line(s) will be tested for viral and bacterial contamination. When proven "clean," the line(s) will be expanded, in vivo or in vitro as appropriate, for large batch cryopreservation. Viability and growth of frozen tumors will be evaluated. The tumors will be included in the Repository's inventory, and upon joint approval of the submitting investigator and the Project Officer; they will be made available for distribution to the scientific community.

MOUSE TUMORS FROM THE JACKSON LABORATORY: These tumors formerly were maintained and distributed by the Jackson Laboratory. The list of available tumors begins on page 42 of the DCTD Tumor Repository Catalog. They were Cryopreserved at EG&G Mason Research Institute and are distributed only as vials of frozen tumor tissue. The required host animals for carrying the JAX tumors in serial transplantation may be obtained from:

Animal Resources
The Jackson Laboratory
600 Main Street
Bar Harbor, NE 04609
USA

T: 800.422.MICE
207.288.5845
F: 207.288.6150

FACTORS OF TRANSPLANTATION LOGISTICS: Since this inventory is concerned with transplantable animal and human tumor systems, the following information must be taken into consideration when planning studies involving tissues to be removed from cryopreservation:

a. Tumors have characteristic lag times (the time lapses between tissue implantation and the first palpable growths), which vary from several days to several months with different tumor systems.
b. Tumors also have characteristic rates of growth which markedly influence host survival, and which may vary from weeks to months with different tumor systems.
c. The above two factors are significantly prolonged in the first, and sometimes the second, transplant generation's post-freeze and thaw.
d. Histologically more complex tumors require two or three transplant generations, after thawing, before they return to normal histology and growth characteristics.

FREEZING PROCEDURE

Solid tumors are frozen as 2 x 2 x 2 mm fragments suspended in a freezing medium. Ascites or tissue-cultured cells are frozen as single cell suspension at a concentration of $10^6 - 10^7$ cells per ml. The freezing medium consists of appropriate tissue culture growth medium plus 10% DMSO and 10% fetal bovine serum.

Aseptically harvested ascites tumors are diluted in the freezing medium at a concentration of $10^6 - 10^7$ cells per ml. One ml suspension is pipetted into each 2 ml vial (Nunc cryotube). The vials are screw-capped tightly and labeled with a Repository number. Tissue cultured cells are prepared in a similar manner. For solid tumors, the aseptically excised tumor tissue is cut into 2 x 2 x 2 mm fragments after freeing it of necrotic materials. The fragments are placed in vials containing l.5 ml of freezing medium.

The processed tumors are frozen initially in a controlled slow-rate freezing apparatus at the rate of 0.5°C per minute to –20°C and 1°C per minute to -80°C. The frozen vials are stored in specific locations in the liquid nitrogen freezers in the Repository after the controlled freezing cycle.

RECOMMENDED THAWING PROCEDURE

Frozen tumor cells or tissues received from the Repository should be kept frozen at -70°C or lower until ready for use. For prolonged storage (more than two days), liquid nitrogen freezers are recommended.

The vials in which the cell lines are stored are reliable; however, they are very susceptible to contamination if thawed in a contaminated water bath. Thawing should be rapid, i.e., within 60-90 seconds. Place the vial in a warm water bath at 37-40°C and agitate vigorously to thaw. Immerse the vial in 70% ethanol before uncapping.

Implant immediately after thawing. The concentration of DMSO is not toxic to tissues and implantation may be made directly from the vial.

For tissue culture, transfer the contents of the vial into a petri dish or flask containing at least 10 volumes of the recommended culture medium and incubate. In order to remove the protective freezing additive (DMSO) from the culture medium, we suggest that the culture medium be changed 24 hours after seeding. If it is desired that DMSO be removed immediately, centrifuge the diluted suspension at approximately 125 X g for 10 minutes, discard the supernatant, and resuspend the cells in an appropriate volume of growth medium.

CAUTION: We strongly recommend wearing protective glasses or face shields when thawing tissues in glass vials.

TUMOR TRANSPLANTATION: It is recognized that transplantable tumor systems are experimental tools for investigators in scientific disciplines other than tumor biology or transplantation immunogenetics. Therefore, we encourage investigators with limited transplantation experience not to hesitate to contact the Tumor Bank for more detailed information on techniques. The following instructions may prove helpful.

TRANSPLANTATION TECHNIQUES: All equipment used should be sterile. Sacrifice the donor animal in a humane manner and immerse it totally in 0.1% Zephiran (Benzalkonium Chloride) or equivalent antiseptic solution.

A. **SUBCUTANEOUS TROCAR IMPLANTATION OF FRAGMENTS** – The tumors are implanted in the axillary region with puncture in the inguinal region. The sacrificed donor animal is pinned to a dissecting board, dorsal surface up. A midline incision is made through the skin, then horizontal incisions at the level of the fore and hind legs are made on the side, usually the right side, where the tumor was implanted. The flap of skin is pinned back, exposing the tumor mass. Free the tumor with scissors or forceps and transfer in to a sterile petri dish placed over ice. Free it of normal fascia and debride it of any necrotic material. Cut the tumor into cubes, usually about 2 x 2 x 2 mm. Thirteen gauge trocars are used to implant the tumors. Lubricate the Trocar by drawing up and expelling some sterile, balanced salt solution, e.g., Earle's, Tissue Culture Medium 199, Hank's etc. With forceps, place a fragment of tumor into the bevel end of the trocar. With a second person holding the animal into which the tumor will be implanted, swab the fur and skin in the area of implant with 70% ethanol. Pierce the skin in the inguinal area with the tip of the trocar, push the trocar forward to the axillary area and eject the tumor fragment from the trocar by pushing the plunger as far into the barrel as possible. In order to prevent the tumor fragment from being pulled back when the trocar is removed, grasp the tip of the trocar through the skin, and then remove the trocar. No more than 30 minutes should elapse from the time the tumor is removed from the donor until it is transplanted.

TROCARS FOR SUBCUTANEOUS IMPLANT OF FRAGMENTS ARE AVAILABLE FROM:

Popper and Sons, Inc.	NAME: Cancer Implant Needle Sets	
300 Denton Avenue	CATALOG NO:	7927
New Hyde Park, New York 11040	DESCRIPTION:	13 G X 3¼"
Telephone: (516) 248-0300	BRAND:	Perfektum

B. **PREPARATION OF TUMOR BREI** – Tumor brei is implanted with a syringe and 19-gauge or smaller needle. It may be implanted subcutaneously, intramuscularly, or intracerebrally. The tumor is excised, freed of normal fascia and debrided of necrotic tissue as described below. The tumor is minced with scissors and pressed through a sterile, 40-gauge stainless steel mesh into a sterile beaker in an ice bath. Add sterile, balanced salt solution, usually in an amount sufficient to prepare a 5% suspension. If specific numbers of cells are to be implanted, a sample of this brei can be counted in a hemocytometer and the brei diluted with sterile balanced salt solution as required. The usual inoculum size is o.2 or 0.3 ml per animal. No more than 30 minutes should elapse from tumor removal to transplantation in all recipient animals.

C. **ASCITES TUMORS** – These are implanted in the peritoneal cavity. Sacrifice and
Disinfect the donor animal as described above. Pin to a dissecting board, ventral surface up. Remove skin from the abdominal area. Withdraw fluid with a sterile syringe by inserting the needle through the abdominal wall. Transfer the fluid from the syringe to a sterile glass container in an ice bath. A cell count may be made, using a hemocytometer. Dilution is made with sterile, balanced salt solution. The usual mouse inoculum is 10^5 cells in 0.1 ml. It is injected intraperitoneally with a 23-gauge needle. No more than 60 minutes should elapse from the time fluid is taken from the donor and stored in the ice bath, and the diluted fluid is implanted in all the recipient animals.

BACTERIAL AND VIRAL MONITORING OF CRYOPRESERVED TISSUES: In addition to testing all freeze-runs for bacterial contamination, Cryopreserved tissues are tested for viral contamination by the MAP test. The viruses tested for are as follows: pneumonia virus of mice (PVM), reo virus-type 3 (Reo 3), Theiler's virus, Murine encephalitis (GD VII), polyoma (Poly), Sendia virus (Send), ectromelia, mouse pox (Ectro), lactic dehydrogenase virus (LDH).

IN VITRO ESTABLISHED CELL LINES:

A. **QUALITY CONTROL AND CHARACTERIZATION** – The quality control and characterization procedures for the incorporation of new cell lines into the Tumor Bank are as follows: Upon receipt, each cell line is immediately transferred to fresh antibiotic free medium and cultured for one week, after which it is tested for mycoplasma (PPLO) contamination. Standard culture

procedures under aerobic and anaerobic conditions, as well as the orcein staining procedure of Fogh, are used. The PPLO medium is extremely rich, and this procedure will also detect most bacterial and fungal contaminants.

B. **FREEZING AND STORAGE** – The cell cultures are frozen in ampules containing 1.0 ml of cell suspension at 2-6 x 10^6 cells/ml in fresh culture medium containing 10% DMSO. Freezing is performed as on page 2. Twenty-four hours after freezing, a representative ampule is removed, thawed, and viable cell count is performed, using the trypan blue dye exclusion procedure. The culture is also tested for its ability to initiate a heavy viable culture. Cell preparations, which show less than 50% viability or poor growth, are discarded and a new lot is prepared.

RECOMMENDED PROCEDURE FOR THAWING FROZEN CELL CULTURES

The vials in which the cell lines are stored are reliable; however, they are very susceptible to contamination if thawed in a contaminated water bath. The following procedures are provided to eliminate this problem.

Remove the ampule form the dry ice container and place it directly into a 37-40°C-water bath (or vessel) of freshly drawn water containing an effective concentration of disinfectant. The thawing should be vigorous and rapid (within 40-60 seconds). As soon as the thawing is complete, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point should be carried out under strict aseptic conditions in a sterile room, cubicle, or hood.

Transfer the contents of the ampule (1 ml volume) into a 100 mm petri dish or 25-cm2 flask containing 8-10 ml of the recommended culture medium, and incubate at the appropriate temperature and carbon dioxide level.

In order to remove the protective freezing additive (DMSO) from the culture medium, we suggest that the culture medium be changed 24 hours after thawing. If it is desired that the freezing additive be removed immediately or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid, and resuspend the cells in an appropriate volume of growth medium.

Subject: Annual Report & Audit Committee meeting
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Thu, 11 Feb 2010 12:51:52 -0500
To: Jane C O'Brien <jobrien@uwo.ca>, Jennifer Stanley <jstanle2@uwo.ca>

Hi Jane

Please find attached the annual report, with information highlighted that you may find useful for the meeting on Tuesday. The yellow sections represent key highlights. I tried to pick both reactive and proactive parts of the report:

Reactive:
- MoL blitz and reporting of injuries to non-workers
- Lab Safety program focus for next year on chemical labelling/segregation, ICFAR...
- workplace violence/harassment part of OHS Act (compliance by June 15, 2010)

Proactive:
- JOHSC inspection review
- respirator fit-tests to prepare the community for the pandemic
- training to be developed for new arc flash standard

I think we should also be prepared to answer questions on the purple highlights...but as you mentioned it is hard to know what might come up!

Feel free to use this information if it is at all helpful to you!

Regards
Jennifer

Safety Annual Report 2009 -with highlights.doc **Content-Type:** application/msword
Content-Encoding: base64

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

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

Completed forms are to be returned to Occupational Health and Safety, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: Paula Foster
SIGNATURE: Paula Foster
DEPARTMENT: Medical Biophysics, Robarts-Imaging
ADDRESS: Imaging Research Labs, RRI.
PHONE NUMBER: x34040
EMAIL: pfoster@imaging.robarts.ca

Location of experimental work to be carried out: Building(s) RRI, UH Room(s) 2-25, 3T, UH1.5T, 2-11

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: OICR, CIHR, NCI, US-DOD,
GRANT TITLE(S): See Attached.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

- ✓ Elizabeth Dunn
- ✓ Paula Foster
- Shruti Krishnamoorthy
- ✓ Lisa Bernas
- Jennifer Noad
- ✓ Laura Gonzalez-Lara
- ✓ Jon Snir
- Vasiliki Economopoulos
- Roja Rohani
- Yuhua Chen
- Mevan Perera

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

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES ⊘ NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ⊘ YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ⊘ No		Not applicable
Rodent	O Yes ⊘ No		
Non-human primate	O Yes ⊘ No		
Other (specify)	O Yes ⊘ No		

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	X Yes O No	See attached Sheet.	
Rodent	X Yes O No		
Non-human primate	O Yes X No		
Other (specify)	O Yes X 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 HC or CFIA containment level required X 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES X 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 Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

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? O YES O NO If no, please proceed to Section 5.0
Already Done.

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
	(4) pCMV-DSRedExpress (5) pGL4.14		RFP LUC	RFP expressed Luciferase expressed

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

Modifications not done by us. Or our lab.

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

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ⊗ NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO n/a

4.6 Will virus be infectious to humans or animals? O YES O NO n/a

4.7 Will this be expected to increase the containment level required? O YES ⊗ NO n/a

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⊗ NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used Nu/Nu (nude) mice, C57Bl/6 mice

6.3 AUS protocol # 2006-013-03, 2007-034-03, 2008-008-01

6.4 Will any of the agents listed be used in live animals ⊗ YES, specify: ALL O NO

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? ⊘YES O NO If no, please proceed to Section 9.0

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

8.3 What is the LD_{50} (specify species) of the toxin 18µg/kg (mice) (no information for humans

9.0 Insects Requiring CFIA Permits

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

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ ⊗ NO
If no, please proceed to Section 10.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE _____

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

13.0 Containment Levels

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

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

☒ YES, permit # if on-campus BIO-RRI-0032 ✓✓

☐ NO

☐ NOT REQUIRED

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Dale Foth Date: Nov 19, 2008

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidder
Date: 19 Dec. 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: Dec 19/08

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

Approval Number: BIO-RRI-0032 Expiry Date (3 years from Approval): Dec 19, 2011

Special Conditions of Approval:

Contact Anne Marie McCusker regarding use of nanoparticles. (X84741)

Biosecurity requirements for pertussis toxins - see www.uwo.ca/humanresources/biosafety (also see attached)

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

Biohazardous Agent Registry Form – P. Foster, November, 2008

Funding Agencies and Grant Titles

- Ontario Institute for Cancer Research: One Millimetre Cancer Challenge (1mmCC), "Tracking Cancer Vaccines"
- National Cancer Institute of Canada: Early and Targeted Detection of Metastatic Cancer in the Lymphatic System Using Cellular Magnetic Resonance Imaging and Nanotechnology

- US Army Department of Defense, Breast Cancer Research Program: Development and Use of Magnetic Resonance Imaging Approaches to Study Dormancy and Metastatic Growth in Preclinical Models of Breast Cancer Metastasis to Brain: Molecular Contributors and Therapeutic Strategies

- Canadian Institutes of Health Research: Development of Cellular Magnetic Resonance Imaging Techniques
- Canadian Institutes of Health Research: Cellular Magnetic Resonance Imaging of Inflammation in Spinal Cord Injury Using Magnetic Nanoparticles

Brief Description Of The Use of the Biohazardous Agents

Cell Lines:
All cell lines will generally be treated in the same fashion. Cells will be subcultured aseptically in a biological cabinet. When they are to be used in an animal experiment, they will be labeled with iron particles (or not, in the case of unlabeled control cells), harvested, diluted to an appropriate concentration then injected into the animals. Methods of injection include subcutaneous, intranodal (lymph node), intracardial, and intravenous. The injections will take place in room 2-25 with the exception of nude mouse injections, which will be done in the Chambers lab in the Medical Biophysics Department at UWO (Med Sci 4th floor) in a sterile cabinet. The mice will then be housed at ACVS and periodically scanned with MRI. MRI scanning may be done at RRI 3T MRI facility, or at University Hospital's 1.5T MRI facility. After reaching end points, animals will be sacrificed, tissues of interest removed, and placed into formalin for further ex vivo scanning or tissue processing. Remaining tissues will disposed of at the ACVS facility for incineration.

Pertussis Toxin:
Pertussis Toxin will be administered to mice with experimental autoimmune encephalomyelitis (EAE). Pertussis Toxin is causes proliferation of T cells, which is important in the induction process of EAE in mice. The toxin, which is missing a subunit resulting in extremely low toxicity in humans, is injected by way of an intraperitoneal injection. The injection will be done by an experienced member of the lab. An SOP has been created. Animals will be housed and imaged as above.

Section 2.3 Biohazardous Agent Registry Form

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

(1) Name: THP-1 (Human)	Supplier: ATCC (TIB-202)
(2) Name: B16F10 (Rodent)	Supplier: Anne Chambers LRCC (collaboration)
(3) Name: Glioma-261 (Rodent)	Supplier: National Cancer Institute (NCI)
(4) Name: Glioma-261 RFP (Rodent)	Supplier: NCI
(5) Name: FaDu Luc2.11 (Human)	Supplier: Becton Dickinson Technologies (collaboration) (Purchased from ATCC (HTB-43) before modification done by BDT)
(6) Name: MDA-MB-231PA(Human)	Supplier: Anne Chambers LRCC (collaboration) (HTB-26)
(7) Name: MDA-MB-231BR(Human)	Supplier: Anne Chambers LRCC (collaboration) (HTB-26)

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:	**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

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

ATCC® Number:	**TIB-202™** Order this Item	**Price:**	**$244.00**
Designations:	THP-1	**Depositors:**	S Tsuchiya
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	monocyte
Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;		
Cellular Products:	lysozyme [58053]		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
	Related Cell Culture Products		
Applications:	transfection host (Technology from amaxa Roche FuGENE® Transfection Reagents)		
Receptors:	complement (C3), expressed [58053] Fc, expressed		
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16		
Age:	1 year infant		
Gender:	male		
Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [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		

Product Description

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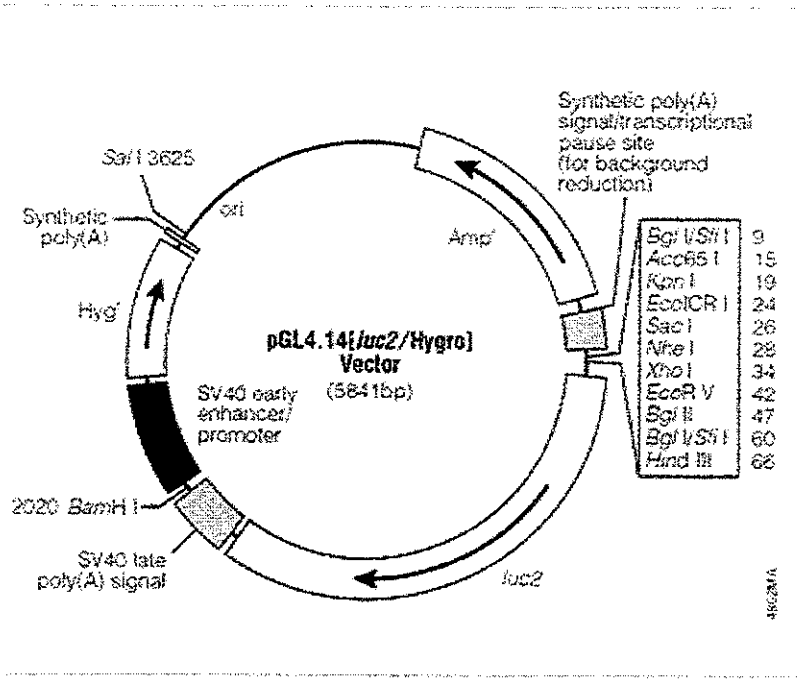
Print this Page

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 arrays 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		

BD	FaDu*Luc*2.11	CONFIDENTIAL
Date: October 30, 2007	Created by: Scott Kaestner	Page 1 of 1

Cell Line	FaDuLuc2.11
Description	The FaDu (human, pharynx squamous cell carcinoma) cell line was established from a punch biopsy of a hypo pharyngeal tumor removed from a Caucasian male. This cell line is adherent and tumorigenic in nude mice. The cell line was transfected with a plasmid containing the Luc2 and Hygromycin resistance genes making the cells bioluminescent in the presence of Luciferin and resistant to Hygromycin. A FaDuLuc2.11 cell contains about 0.3 pg of Luciferase. FaDu cells stain positive with Pancytokeratin immuno histochemical staining. FaDu cells over express Epithelial Growth Factor Receptor.
Media and sub cultivation	RPMI-1640 with L-glutamine, 10% Fetal Bovine Serum, 10,000 IU/ml penicillin, 10,000µg/ml streptomycin, and 150 µgs/ml Hygromycin. Cryoprotectant media is the same as above supplemented with 5% DMSO. A sub cultivation ratio of 1:3 to 1:6 is best for this cell line and is compatible with a bi-weekly media replacement and passaging regiment.
Tumorigenicity	Primary subcutaneous tumor: 5/5 (100%), $1.0x10^6$ cells inoculated, Bioluminescent detection at day 7, palpable at day 14. Intranodal tumor: 11/19 (58%), $5.0x10^4$ cells inoculated, Bioluminescent detection at day 1 and 5.
MTA and other IP	The original FaDu cell line was purchased from ATCC and may be subject to terms from the Material transfer agreement at time of purchase. The Promega pGL4.14 vector is the primary backbone of the plasmid used for transfection of the FaDu cell line and some genes may be patent protected.



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