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): _____

Chair,Biohazards Subcommittee: _____

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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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.

email Paula

ViroMed Laboratories
A LabCorp Company

CHUNG 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.

Source -> same 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 P. Forster
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] Horoszewiez, 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:	Fisher
		Cat #:	BW12-741F
		Package size:	500 ml
HAM'S F12	liquid with sodium bicarbonate	Vendor:	Fisher
		Cat #:	BW12-615F
		Package size:	500 ml
FBS	Premium Heat inactivated	Vendor:	Fisher
		Cat #:	BW14-503F
		Package size:	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)

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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 .