Western University
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: April 13, 2012
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

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

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

PRINCIPAL INVESTIGATOR:	**Leonard Luyt**
DEPARTMENT:	**Oncology**
ADDRESS:	**LRCP**
PHONE NUMBER:	**519-685-8600 x53302**
EMERGENCY PHONE NUMBER(S):	**519-282-1665**
EMAIL:	**lluyt@uwo.ca**

Location of experimental work to be carried out :

Building :		Room(s):	
Building :	**LRCP (Dr. Eva Turley lab)**	Room(s):	**A4-931, A4-928, A4-834**
Building :		Room(s):	

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 16.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CBCF**

GRANT TITLE(S):

CBCF
Molecular Imaging of Highly Invasive Breast Cancer Subsets using Novel Peptide Mimetics

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

Name	UWO E-mail Address Only	Date of Biosafety Training
see comment below		

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

CBCF

All biological agents will be used in the lab of Dr. Eva Turley, who is co-applicant on this grant and is under biosafety approval number BIO-LRCC-0006. Personnel working at this location are under the supervision of Dr. Eva Turley and are listed on BIO-LRCC-0006.

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

CBCF

Early detection and tumor cell heterogeneity are two clinical issues that are obstacles to better patient outcome in breast cancer (BCA). Development of biomarkers that can be used to detect early lesions and predict which of these contain aggressive tumor cell subsets are needed for successful treatment. We have linked the presence of hyaluronan (HA) receptors/Texas Red (TR)-HA binding to a BCA subpopulation that is uniquely characterized, in culture and in vivo by its slow proliferation, stem-like surface phenotype (CD44+/RHAMM+/CD24-/ESA+/TR-HA++), resistance to hypoxia and high invasion (e.g. lymphatic invasion index in vivo). Since other laboratories have shown that HA promotes chemoresistance, we predict this BCA subpopulation contributes to therapy resistance and treatment failure. Our hypothesis is that radiolabelled molecular imaging probes mimicking HA will detect this subpopulation and allow early identification of BCA that have the potential to be aggressively invasive and treatment resistant.
We have recently identified small peptides that behave as HA mimetics with a strong affinity for RHAMM (KD <30 nM). Targeting RHAMM is optimal for identifying the above subsets since, unlike CD44, it is not expressed in normal breast tissue. We are developing positron emission tomography (PET) imaging agents based on these peptides. We now propose to: 1) create in vivo stable PET imaging HA mimetic probes; 2) Evaluate the ability of HA peptide mimic PET imaging agents to target CD44+/RHAMM+/CD

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species __________
What is the origin of the microorganism(s)? __________

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. Coli	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.5L@0.70D frozen stock**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

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

Additional Comments: __________

Changes to 1.2

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☒ No		Not applicable
Rodent	☒ Yes ☐ No	**mouse fibroblastic cells** (transfected with Δ1-163 RHAMM cDNA, see section 4.2)	**2009-060, 2009-051**
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**prostate stem cells, MDA-mb-231, MCF7**	**1**	**Dr. Jim McCarthy's lab, U Minnesota ATCC**
Rodent	☒ Yes ☐ No	**Rhamm-/-MEF, CD44-/-MEF, Rhamm-/-CD44-/MEF, 10T1/2**	**1**	**ATCC** **our mice**
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☒ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ NO
If no, please proceed to Section 4.0

Changes to 2.2, 2.3 and 2.4

3.2 Indicate in the table below the Human Source Materia

Human Source Material	**Source/Supplier /Company Name**	**Is Huma Material With An Agent? YES/UNKNOWN**	**applicable)**	**one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction)		☐ Yes		☐1 ☐2

Tissues (unpreserved)		☐ Unknown		☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☒ YES ☐ NO If **NO**, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?	If plasmids are being used to transfect cells what is the consequence on the eukaryotic cells?
E.coli K12 Strains: DH5 alpha XL-1 Blue BI21 HB101 TOP10 All plasmids are propagated in one of these strains	pCDNA3.1	Parental Vector from INVITROGEN. Inserts were generated in laboratory or obtained from ATCC/NCBI Plasmid maps as per BIO-LRCC-0006 (attached).	containing the following inserts: -RHAMM cDNAs from mouse and human. -RHAMM fusion protein vector containing RHAMM fused to ZsGreen coding sequence. - mutant active MEK1 coding sequence from mouse - H-RAS from mouse	-Alterations in cell motility - Transformation of fibroblasts - fluorescent proteins for visualization of protein trafficking			
	pH-Apr-1-Neo pH-Apr-1-Hygro Reference Gunning, Leavitt, Muscat, Ng, Kedes.	Plasma generated by colleague referenced. Neo denotes Neomycin selection cassette and Hygro indicates Hygromycin selection	RHAMM cDNAs from mouse and human	-Alterations in cell motility - Transformation of fibroblasts			

	PNAS 84:4831, 1987.	cassette. Plasmid maps as per BIO-LRCC-0006 (attached).					
	pGEX-2TK	GE healthcare Plasmid maps as per BIO-LRCC-0006 (attached).	- RHAMM cDNAs from mouse and human for recombinant protein production in E.coli strains listed	None. Protein production.			
	pPAL7	Biorad Plasmid maps as per BIO-LRCC-0006 (attached).	- RHAMM cDNAs from mouse and human for recombinant protein production in E.coli strains listed	None. Protein production.			

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

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

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

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

4.3.1 Will virus be replication defective? ☐ YES ☒ NO

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

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

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☐ NO ☒ YES, specify **RHAMM/HMMR**
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

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

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Biological Toxins and Hormones

7.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 8.0

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

7.3 What is the LD_{50} (specify species) of the toxin or hormone

7.4 How much of the toxin or hormone is handled at one time*?

7.5 How much of the toxin or hormone is stored*?

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

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

Additional Comments: _____

8.0 Animal Experiments

8.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 9.0

8.2 List animal species to be used: **Mus Musculus (Mouse)**

8.3 AUS protocol number(s): **2009-060, 2009-051**

8.4 List the location(s) for the animal experimentation and housing: **VH-VRL (7th floor)**

8.5 Will any of the agents listed in Sections 1-7 be used in live animals
☐ NO ☒ YES, specify: **Fibroblasts stably transfected with RHAMM cDNA will be used in Xenograft studies**

8.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: **Cells used in study may form tumours in mammary fat pads, but these are not shed by the animal.**

8.7 Indicate the PHAC or CFIA containment level used: ☐ 1 ☒ 2 ☐ 2+ ☐ 3

9.0 Use of Animal species with Zoonotic Hazards

9.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 10.0

9.2 Will live animals be used? ☐ YES ☐ NO

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

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

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

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

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

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

11.3 What is the origin of the plant?

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

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

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

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

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

12.0 Import Requirements

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

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

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☐
Enter Your Name **Leonard Luyt** ***Date:*** **04Dec2012**

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: 03Dec2012
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **Bio-LRCC-0006**

15.0 Procedures to be Followed

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

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Wash area with soap and water (or use eyewash station if eyes are exposed) and report to Occupational Health.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at [illegible]

Please print and sign this page and submit it. This signature page is needed for approval.

Researcher: SIGNATURE: _____
Date: 04 Dec 2012

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: 12 DEC 2012

-------- Original Message --------
Subject: Re: Fwd: BARF for CBCF team grant
Date: Wed, 12 Dec 2012 13:29:52 -0500
From: Len Luyt <lluyt@uwo.ca>
To: Jamie Gibbings <Jamie.Gibbings@lhsc.on.ca>
CC:jstanle2@uwo.ca

I agree to meet all requirements indicated in section 13.
Len Luyt

On 12/12/2012 1:23 PM, Jamie Gibbings wrote:
Hi Len and Jennifer:

Len - Small formality, however you did not check the box under Section 13. Please reply all stating that you agree to meet all requirements in section 13.

Jennifer - I have attached my signature and approve once you have received Len's reply. Please attach the reply to the document.

13.0 Training Requirements for Personnel Named on Form
All personnel named on the above form who will be using any of the above named agents are required to attend
the following training courses given by OHS:
♦ Biosafety
♦ Laboratory and Environmental/Waste Management Safety
♦ WHMIS (Western or equivalent)
♦ Employee Health and Safety Orientation
As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of
the biological agents in Sections 1.0 to 9.0 have been trained.
An X in the check box indicates you agree with the above statement...
Enter Your Name Leonard Luyt Date: 04Dec2012

Thanks,

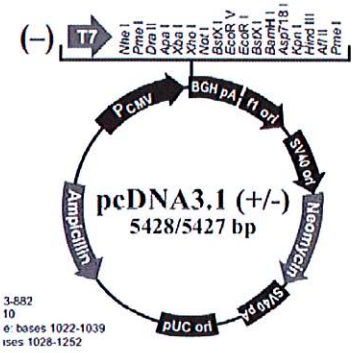
Jamie Gibbings, CRSP
Safety Coordinator
Occupational Health & Safety Services
London Health Sciences Centre
T. 519-685-8500 x77068
P. 15641

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

Special Conditions of Approval:

Source of Plasmid

Parental Vector from INVITROGEN. Inserts were generated in laboratory or obtained from ATCC/NCBI



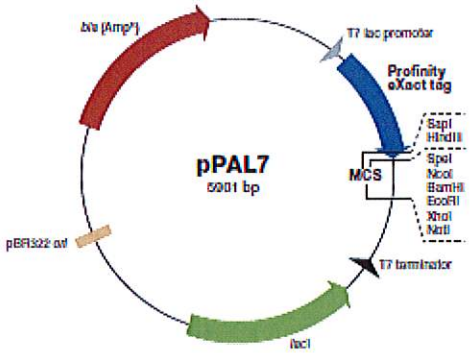
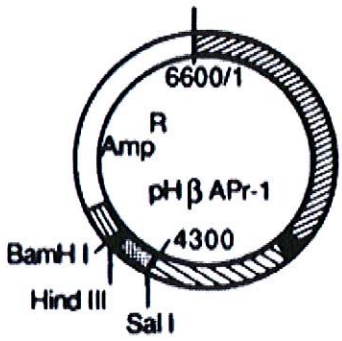
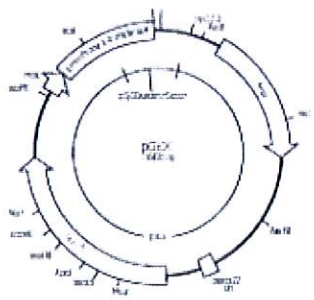


Fig. 1. Profinity eXact pPAL7 vector.

Plasmid map attached. Plasmid generated by colleague referenced. Neo denotes Neomycin selection cassette and Hygro indicates Hygromycin selection cassette



GE-HEALTHCARE



Cell Biology

ATCC® Number:	CRL-1740™ Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price** See New Benefits of ATCC Culture

Designations:	LNCaP clone FGC
Depositors:	JS Horoszewicz
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent, single cells and loosely attached clusters
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** left supraclavicular lymph node
Cellular Products:	human prostatic acid phosphatase; prostate specific antigen
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1977
Applications:	transfection host
Receptors:	androgen receptor, positive; estrogen receptor, positive [23045]
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 10,12 D16S539: 11 D5S818: 11,12 D7S820: 9.1,10.3 THO1: 9 TPOX: 8,9 vWA: 16,18
Cytogenetic Analysis:	This is a hypotetraploid human cell line. The modal chromosome number was 84, occurring in 22% of cells. However, cells with chromosome counts of 86 (20%) and 87 (18%) also occurred at high frequencies. The rate of cells with higher ploidies was 6.0%.
Age:	50 years adult
Gender:	male
Ethnicity:	Caucasian

Related Links ▸

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Material Transfer Agreement New!
Technical Support
Related Cell Culture Products
Product Information Sheet

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

Comments:	LNCaP clone FGC was isolated in 1977 by J.S. Horoszewicz, et al., from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old Caucasian male (blood type B+) with confirmed diagnosis of metastatic prostate carcinoma. These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production). The cells do not produce a uniform monolayer, but grow in clusters which should be broken apart by repeated pipetting when subcultures are prepared. They attach only lightly to the substrate, do not become confluent and rapidly acidify the medium. Growth is very slow. The cells should be allowed to incubate undisturbed for the first 48 hours after subculture. When flask cultures are shipped, the majority of the cells become detached from the flask and float in the medium. Upon receipt, incubate the flask (in the usual position for monolayer cultures) for 24 to 48 hours to allow the cells to re-attach. The medium can then be removed and replaced with fresh medium. If desired, the contents of the flask can be collected, centrifuged at 300 X g for 15 minutes, resuspended in 10 ml of medium and dispensed into a single flask.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Maintain cultures at a cell concentration between 1 X 10(4) and 2 X 10(5) cells/cm2. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	about 34 hours
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-10995 purified DNA:ATCC CRL-1740D

References:

21889: . Models for prostate cancer. 37New York: Liss; 1980.
22410: Gibas Z, et al. A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet. Cytogenet. 11: 399-404, 1984. PubMed: 6584201
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Designations:	PC-3
Depositors:	ME Kaighn
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent (The cells form clusters in soft agar and can be adapted to suspension growth)
Organism:	*Homo sapiens*
Morphology:	epithelial PHOTO
Source:	**Organ:** prostate **Tumor Stage:** grade IV **Disease:** adenocarcinoma **Derived from metastatic site:** bone
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Tumorigenic:	Yes
Antigen Expression:	HLA A1, A9
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 11 D16S539: 11 D5S818: 13 D7S820: 8,11 THO1: 6,7 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	The line is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.
Age:	62 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:

Protocol:

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

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
recommended serum:ATCC 30-2020

References:

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Designations:	DU 145
Depositors:	KR Stone
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** brain
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Tumorigenic:	Yes
Antigen Expression:	Blood Type O; Rh+
DNA Profile (STR):	D7S820: 7, 10, 11 D13S317: 12, 13, 14 D5S818: 10, 13 vWA: 17, 18, 19 THO1: 7 CSF1PO: 10, 11 TPOX: 11 D16S539: 11, 13 Amelogenin: X, Y
Cytogenetic Analysis:	This is a hypotriploid human cell line. Both 61 and 62 chromosome numbers had the highest rate of occurrence in 30 metaphase counts.The rate of higher ploidies was 3%. The t(11q12q), del(11)(q23), 16q+, del(9)(p11), del(1)(p32) and 6 other marker chromosomes were found in most cells. The N13 was usually absent. The Y chromosome is abnormal through translocation to an unidentified chromosomal segment. The X chromosome was present in single copy.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 1-2 PGM1, 1 PGM3, 2
Age:	69 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The line is not detectably hormone sensitive, is only weakly positive for acid phosphatase and isolated cells form colonies in soft agar. The cells do not express prostate antigen. Ultrastructural analyses of both the cell line and original tumor revealed microvilli, tonofilaments, desmosomes, any mitochondria, well developed Golgi and heterogenous lysosomes.
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%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:

Protocol:

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

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium, 95%; DMSO, 5%
Storage temperature: liqid nitrogen vapor temperature

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-81D
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

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Designations:	MDA-MB-231
Depositors:	R Cailleau
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
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.
Applications:	transfection host
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
Gender:	female
Ethnicity:	Caucasian
Comments:	The cells express the WNT7B oncogene [PubMed: 8168088].
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 100% **Temperature:** 37.0°C

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Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C without CO2.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2008 recommended serum: ATCC 30-2020 purified DNA: ATCC 45518 purified DNA: ATCC 45519 purified DNA: ATCC HTB-26D purified RNA: ATCC HTB-26R Component of: Triple Negative Breast Cancer Cell Panel, ATCC TCP-1002

References:

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Designations:	HUV-EC-C
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	endothelial
Source:	**Organ:** umbilical vein **Tissue:** vascular endothelium **Disease:** normal **Cell Type:** endothelial
Cellular Products:	factor VIII
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 9,11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,11 vWA: 16
Cytogenetic Analysis:	Karyology performed for one batch of CRL-1730 in 1996 reflected a hypodiploid human cell line with a modal chromosome number of 45 occurring in 72% of the cells counted, all of which had monosomic N13. The rate of polyploid cells among this population was 15.8%. This karyology differed from earlier work-ups performed on the cells that showed approximately 60% of the cells retained 2 chromosomes 13. The apparent clonal variation in cultures of CRL-1730 (most likely dependent upon passage and growth conditions) has also been noted in STR profiles with unstable alleles at D13S317 allele #9, D13S317 allele #11, and D7S820 allele #12. Other coexisting subclones include those with 46,XX,-11,-13,i(11p),i(11q) and 46,XX,+11,-13 karyotypes. For all karyotypes performed, both X chromosomes appear normal.
Comments:	Endothelial Cell Growth Supplement (ECGS) and unidentified factors from bovine pituitary, hypothalamus or whole brain extracts are mitogenic for this line. The cells have a life expectancy of 50 to 60 population doublings.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated of F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: 0.1 mg/ml heparin; 0.03-0.05 mg/ml endothelial cell growth supplement (ECGS); adjust to a final concentration of 10% fetal bovine serum. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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Subculturing:	**Note:** A high quality ECGS prepared from bovine neural tissue (Sigma Cat no. E-2759 or equivalent) should be used to propagate CRL-1730. It is best to initiate the cells with the highest recommended concentration of ECGS.**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended **Medium Renewal:** Two to three times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020
References:	23284: Hoshi H, McKeehan WL. Brain- and liver cell-derived factors are required for growth of human endothelial cells in serum-free culture. Proc. Natl. Acad. Sci. USA 81: 6413-6417, 1984. PubMed: 6333682 29192: Zahedi K. Characterization of the binding of serum amyloid P to laminin. J. Biol. Chem. 272: 2143-2148, 1997. PubMed: 8999915 33021: Soker S, et al. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain. J. Biol. Chem. 271: 5761-5767, 1996. PubMed: 8621443

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ATCC® Number:	CCL-61™ Order this Item	Price:	$431.00 (for-profit list price) $359.17 (non-profit list price) Log In with customer # to see your price See New Benefits of ATCC Culture

Designations:	CHO-K1
Depositors:	TT Puck
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cricetulus griseus*
Morphology:	epithelial-like PHOTO
Source:	**Organ:** ovary
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:** 1957
Applications:	transfection host
Virus Resistance:	poliovirus 2; modoc virus; Button Willow virus
Cytogenetic Analysis:	Chromosome Frequency Distribution 50 Cells: 2n = 22. Stemline number is hypodiploid.
Gender:	female
Comments:	The CHO-K1 cell line was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T. T. Puck in 1957. The cells require proline in the medium for growth.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended **Medium Renewal:** Once or twice between subculture
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020

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32522: Siess DC, et al. Exceptional fusogenicity of chinese hamster ovary cells with murine retrovirus suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process. J. Virol. 70: 3432-439, 1996. PubMed: 8648675
32691: Wang H, et al. Modulation of ecotropic murine retrovirus by N-linked glycosylation of the cell surface receptor/amino acid transporter. J. Virol. 70: 6884-6891, 1996. PubMed: 8794331
32720: Chen Y, et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005
32833: Nohturfft A, et al. Recurrent G-to-A substitution in a single codon of SREBP cleavage-activating protein causes sterol resistance in three mutant chinese hamster ovary cell lines. Proc. Natl. Acad. Sci. USA 93: 13709-13714, 1996. PubMed: 8942999
32908: Blanke SR, et al. Fused polycationic peptide mediates delivery on diphtheria toxin A chain to the cytosol in the presence of anthrax protective antigen. Proc. Natl. Acad. Sci. USA 93: 8437-8442, 1996. PubMed: 8710889
32911: Efthimiopoulos S, et al. Cholinergic agonists stimulate secretion of soluble full-length amyloid precursor protein in neuroendocrine cells. Proc. Natl. Acad. Sci. USA 93: 8046-8050, 1996. PubMed: 8755600
32918: Sharma A, et al. Reduction in the level of Gal(alpha 1,3)Gal in transgenic mice and pigs by the expression of an alpha(1,2)fucosyltransferase. Proc. Natl. Acad. Sci. USA 93: 7190-7195, 1996. PubMed: 8692967
33011: Ozcelebi F, et al. Phosphorylation of cholecystokinin receptors expressed on chinese hamster ovary cells. J. Biol. Chem. 271: 3750-3755, 1996. PubMed: 8631990
33028: Lee RJ, Huang L. Folate-targeted, anionic liposome-entrapped polylysine-condensed DNA for tumor cell-specific gene transfer. J. Biol. Chem. 271: 8481-8487, 1996. PubMed: 8626549
33052: Hawes BE, et al. Phosphatidylinositol 3-kinase is an

Western

Researcher: Dr. E. Turley

Biosafety Approval Number: BIO-LRCC-0006

Expiry Date: November 23, 2014

November 29, 2011

Dear Dr. Turley:

Please note your biosafety approval number listed above. This number is very useful to you as a researcher working with biohazards. It is a requirement for your research grants, purchasing of biohazardous materials and Level 2 inspections.

Research Grants:

- This number is required information for any research grants involving biohazards. Please provide this number to Research Services when requested.

Purchasing Materials:

- This number must be included on purchase orders for Level 1 or Level 2 biohazards. When you order biohazardous material, use the on-line purchase ordering system (www.uwo.ca/finance/people/). In the "Comments to Purchasing" tab, include your name as the Researcher and your biosafety approval number.

Annual Inspections:

- If you have a Level 2 laboratory on campus, you are inspected every year. This is your permit number to allow you to work with Level 2 biohazards.

To maintain your Biosafety Approval, you need to:

- Ensure that you update your Biohazardous Agents Registry Form at least every three years, or when there are changes to the biohazards you are working with.
- Ensure that the people working in your laboratory are trained in Biosafety.
- Ensure that your laboratory follows the University of Western Ontario Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories.
- For more information, please see: www.uwo.ca/humanresources/biosafety.

Please let me know if you have questions or comments.

Regards,

Jennifer Stanley

Jennifer Stanley
Biosafety Coordinator for Western
Stevenson Lawson Building Room 295G
Phone: 519-661-2111 X81135
Fax: 519-661-3420

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	Dr. Eva Turley
DEPARTMENT	Oncology, Cancer Research lab, LRCP
ADDRESS	790 Commissioners Rd. E. Room A4-931
PHONE NUMBER	519-685-8500 Ex. 53677 Lab Ex.53280
EMERGENCY PHONE NUMBER(S)	519-685-8500 Ex. 53280
EMAIL	Eva.Turley@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LRCP building A Room(s)_A4-931, A4928, A4-824

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

FUNDING AGENCY/AGENCIES: CBCF, NSERC, MITACS (Octoderma)

GRANT TITLE(S):
1. RHAMM / HMMR in Breast and Prostate Cancer progression
2. The role of RHAMM in wound repair.
3. Unconventional export and trafficking of RHAMM

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

Name	UWO E-mail Address	Date of Biosafety Training
Jenny Ma	Jenny.Ma@lhsc.on.ca	Jun 23, 2011
Conny Toelg	Conny.Toelg@lhsc.on.ca	Jun 24, 2011
Natalia Akentieva	Natalia.Akentieve@lhsc.on.ca	Jun 23, 2011
Pat Telmer	Patrick.Telmer@lhsc.on.ca	Jun 24, 2011
Siddika Pardhan	spardha@uwo.ca	Jun 23, 2011

Sallie Elhayek	selhayek@uwo.ca	Jun 24,2011

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

1. We use *E.coli* strain K12 bacteria for propagation of protein expression plasmids and for producing recombinant RHAMM proteins. We use these proteins as reagents for producing polyclonal antibodies, as function blockers of cell invasion/migration and proliferation in culture and *in vivo*, for characterizing binding characteristics *in vitro* and for screening peptide mimetic/small chemical libraries to identify Rhamm function blocking reagents.
   All reagents for bacterial work are stored in labeled areas of the laboratory and cold room. Liquid bacterial cultures are sterilized with bleach overnight before disposing. All bacterial plates and contaminated pipettes are disposed of in Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies.

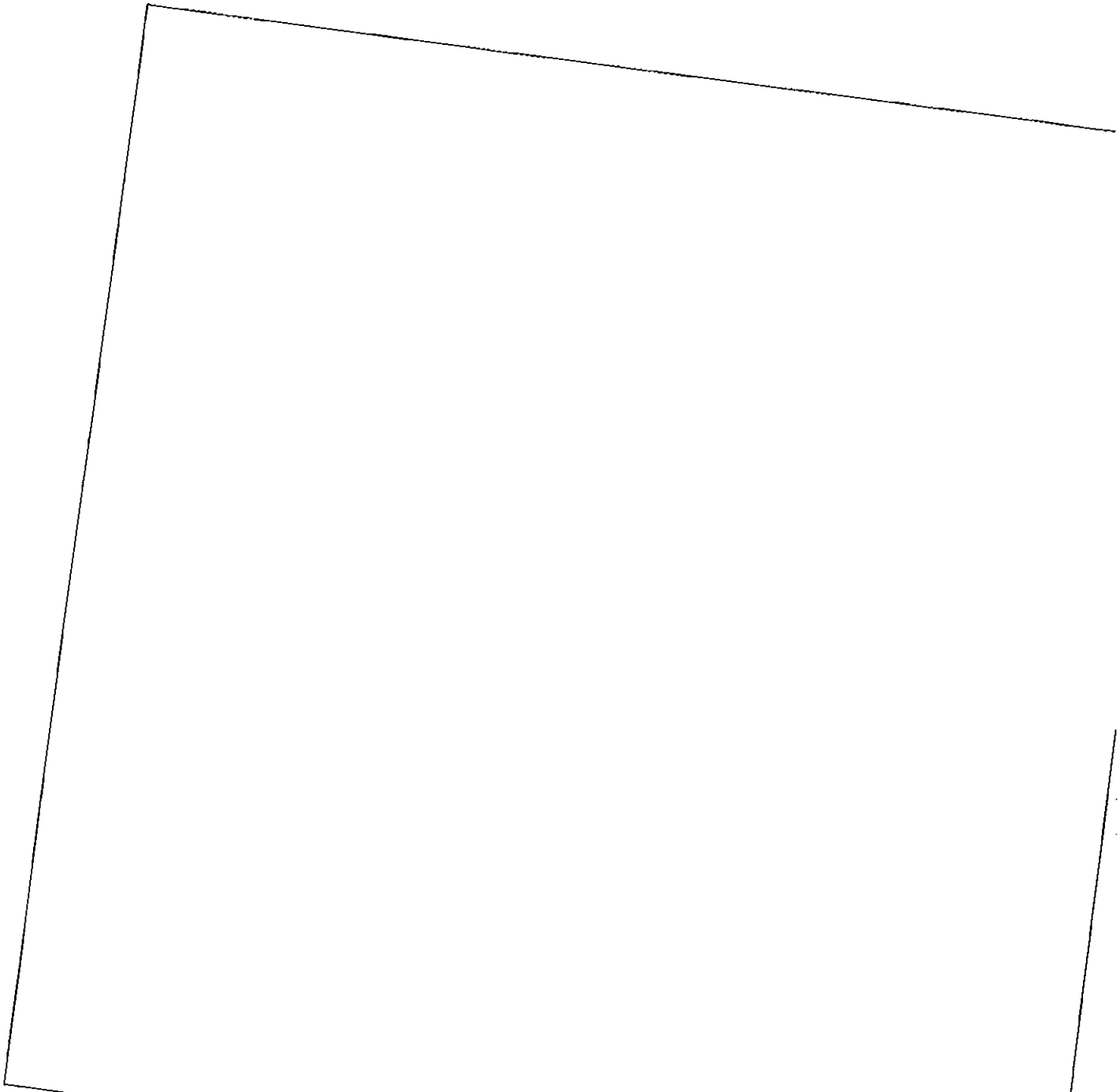
2. We use human cell lines including fibroblast, breast cancer, prostate cancer, and melanoma obtained from ATCC for in culture and *in vivo* (immune compromised mice) experiments.
   All cell culture reagents are disposed of Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies. Liquid Media is decontaminated with bleach overnight before disposal. Frozen cell lines are kept in -80, -150 freezers or liquid nitrogen tank that are clearly labeled.

3. We have several varieties of rodent cells which we use to assess the function and localization of Rhamm and to characterize the effects of blocking Rhamm function on cell migration/proliferation. We use murine fibroblast lines obtained from ATCC along with primary cultures which are prepared by our lab. The primary cultures are muring embryonic fibroblasts, dermal fibroblasts, wound site fibroblasts, and bone marrow fibroblasts (stem cells). These are grown from explanted tissue and maintained in culture for approximately 8 passages. We obtain these primary cells from wildtype BL6 mice, Rhamm -/- BL6 mice, CD44-/- BL6 mice, and Rhamm-/-;CD44-/- BL6 mice.
   All cell culture reagents are disposed of Biohazardous waste containers in strict accordance with London Health Sciences hazardous waste policies. Liquid Media is decontaminated with bleach overnight before disposal.

New Info

Please include a one page research summary or teaching protocol.

My research program currently focuses upon defining the mechanisms by which the polysaccharide hyaluronan controls wound repair and the related process of tumour progression. Hyaluronan is a large negatively charged glycosaminoglycan produced by hyaluronan synthases. It functions both to organize the pericellular matrix and to activate signaling cascades that control cell migration and cell division. My laboratory identified, characterized and cloned the first cellular hyaluronan receptor, termed Rhamm (gene name HMMR). We are investigating the mechanisms by which this intracellular and extracellular protein controls wound repair, mesenchymal stem cell trafficking and breast/prostate cancer progression.



1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES xO 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)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.Coli	xO Yes O No	Ox Yes O No	Ox Yes O No	0.5L@0.70D frozen stock	Invitrogen	xO 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

dec 18/11 per conversation with Jenny ma

*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? xO 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes x O No		Not applicable
Rodent	xO Yes O No	mouse fibroblastic cells	2009-060, 2009-051
Non-human primate	O Yes x O No		
Other (specify)	O Yes ~~O~~ X No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	xO Yes O No	prostate stem cells, MDA-mb-231, MCF7, Melanoma WM1552C, Ovarian cancer SKOV3, OVCAR	2	Dr. Jim McCarthy's lab Minnesota, ATCC
Rodent	xO Yes O No	Rhamm-/-MEF, CD44-/-MEF, Rhamm-/-CD44-/MEF, 10T1/2, C3 10T1/2, MEF Rhamm+MEK, rat dermal fibroblasts, rat mesenchymal stem cells, Raw 264.7	2	Our mice, ATCC
Non-human primate	O Yes xO No			Level 1 per ATCC.org
Other (specify)	O Yes xO No			

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? xO YES O NO
If no, please proceed to Section 4.0

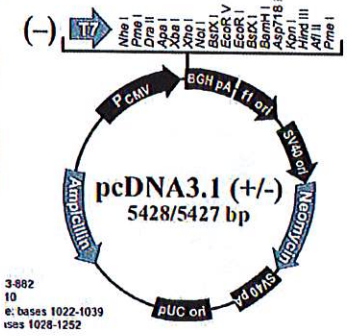
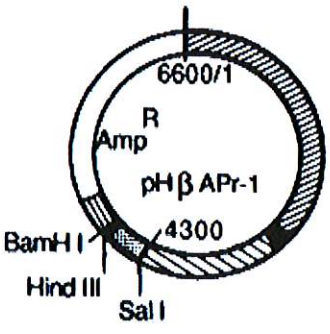
3.2 Indicate in the table below the Human Source Material to be used.

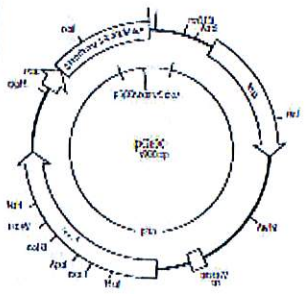
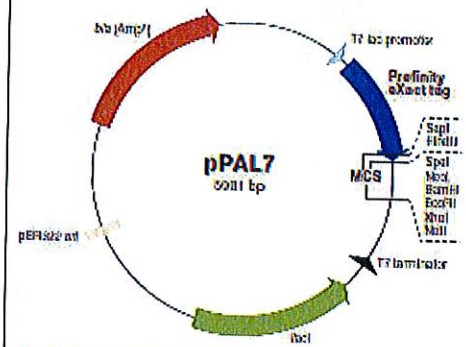
Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	LRCP patients, Dr. Trevor Shepherd	O Yes xO Unknown		xO 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)	Isu Abxis Accumax tissue arrays	Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? xO YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E.coli K12 Strains: *DH5 alpha* *XL-1 Blue* *Bl21* *HB101* *TOP10* *All plasmids are propagated in one of these strains*	*pCDNA3.1*	*Parental Vector from INVITROGEN. Inserts were generated in laboratory or obtained from ATCC/NCBI* 	*containing the following inserts:* *-RHAMM cDNAs from mouse and human.* *-RHAMM fusion protein vector containing RHAMM fused to ZsGreen coding sequence.* *-Mutant – Active MEK1 coding sequence from mouse.* *-H-RAS from Mouse*	*-Alterations in cell motility* *-Transformation of fibroblasts* *-Fluorescent proteins for visualization of protein trafficking*
	pH-Apr-1-Neo *pH-Apr-1-Hygro* *REFERENCE:* Gunning, P., J. Leavitt, G. Muscat, S. Y. Ng, and L. Kedes. 1987. A human beta-actin expression vector system directs high-level accumulation of antisense transcripts. Proc. Natl. Acad. Sci. USA 84:4831–4835.	*Plasmid map attached. Plasmid generated by colleague referenced. Neo denotes Neomycin selection cassette and Hygro indicates Hygromycin selection cassette* 	*-RHAMM cDNAs from mouse and Human*	*-Alterations in cell motility* *-Transformation of fibroblasts*

	pGEX-2TK	*GE-HEALTHCARE* 	*-RHAMM cDNAs from mouse and Human for recombinant protein production in E.coli strains listed*	*None. Protein production*
	pPAL7	*BIORAD*  Fig. 1. Profinity eXact pPAL7 vector.	*-RHAMM cDNAs from mouse for recombinant protein production in E.coli strains listed*	*None. Protein production*

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ xO NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ xO NO
- SV 40 Large T antigen O YES xO NO
- E1A oncogene O YES xO NO
- Known oncogenes xO YES, please specify: RHAMM/HMMR, RAS, MEK1 _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify_____ ~~O~~ NO

4.5 Will virus be replication defective? O YES O NO

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

4.7 Will this be expected to increase the containment level required? O YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES xO NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mus Musculus (Mouse)

6.3 AUS protocol # _2009-060, 2009-051_____

6.4 Will any of the agents listed in section 4.0 be used in live animals xO YES, specify: Fibroblasts stably transfected with RHAMM cDNA will be used in Xenograft studies O NO

6.5 Will the agent(s) be shed by the animal: O YES xO NO, please justify: Cells used in study may form tumours in mammary fat pads, but these are not shed by the animal.

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES XO No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

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

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

_____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____
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_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

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

9.0 Insects

9.1 Do you use insects? O YES xO 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 life stage of the insect? _____

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

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

_____
_____

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

10.0 Plants

10.1 Do you use plants? O YES xO 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
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ x O NO
If no, please proceed to Section 12.0

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 or plant 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
xO YES, date of most recent biosafety inspection: Bio-LRCC-006 Level 2 Biosafety Inspection Dec. 10, 2010 Marie Ryder
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): Bio-LRCC-0006

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
No specific measures beyond standard biosafety associated with levels 1 and 2

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
- Seek first aid or emergent intervention if required (refer to MSDS for Infectious Substances, or Blood-Borne Pathogen exposure protocol: http://intra.sjhc.london.on.ca/policy/search_res.php?polid=STF008&live=1
- Immediately report injury, exposure to supervisor
- Notify Occupational Health and Safety Services (OHSS) ASAP. Complete Workplace Occurrence Report: http://appserver.lhsc.on.ca/policy/search_res.php?polid=OHS011&live=1

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

SIGNATURE _____ Date: June 29 / 11

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 24 Nov 2011

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Nov 18/11

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Marie Ryder
Date: July 7, 2011

Approval Number: BIO-LRCC-0006 Expiry Date (3 years from Approval): Nov 23, 2014

Special Conditions of Approval:

Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

Sincerely,

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

Canada

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Info on Cell Line(s)

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Cell Biology

ATCC® Number: HTB-26™ Order this Item

Price: $279.00

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.

Applications: transfection host (Nucleofection technology from Lonza
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.

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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

Gender: female

Ethnicity: Caucasian

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C without CO2.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: 2 to 3 times per week

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 30-2008
recommended serum:ATCC 30-2020
purified DNA:ATCC 45518
purified DNA:ATCC 45519
purified DNA:ATCC HTB-26D
purified RNA:ATCC HTB-26R

References:

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32272: Katayose Y, et al. Promoting apoptosis: a novel activity associated with the Cyclin-dependent kinase inhibitor p27. Cancer Res. 57: 5441-5445, 1997. PubMed: 9407946
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32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194
32489: De Vincenzo R, et al. Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines. Anticancer Drug Des. 13: 19-33, 1998. PubMed: 9474240
33021: Soker S, et al. Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain. J. Biol. Chem. 271: 5761-5767, 1996. PubMed: 8621443

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Cell Biology

ATCC® Number: HTB-22™ Order this Item

Price: $279.00

Designations: MCF7

Depositors: CM McGrath

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

Cellular Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

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
Roche FuGENE® Transfection Reagents)

Receptors: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

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Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1
Age:	69 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% . **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. **Note:** if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures. 1. Remove culture medium to a centrifuge tube. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant. 6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. 7. Incubate cultures at 37C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	29 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 purified DNA:ATCC HTB-22D purified RNA:ATCC HTB-22R 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X

References:

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38764: Bacus SS, et al. Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. Mol. Carcinog. 3: 350-362, 1990. PubMed: 1980588

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Cell Biology

ATCC® Number: HTB-77™ Order this Item

Price: $279.00

Designations: SK-OV-3 [SKOV-3]

Depositors: G Trempe, LJ Old

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: ovary
Disease: adenocarcinoma
Derived from metastatic site: ascites

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

Restrictions: The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1973

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Tumorigenic: Yes

Antigen Expression: Blood Type B; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 8,11
D16S539: 12
D5S818: 11
D7S820: 13,14
THO1: 9,9.3
TPOX: 8,11
vWA: 17,18

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Cytogenetic Analysis:	This is a hypodiploid human cell line. The modal chromosome number was 43, occurring in 63.3% of cells. The range was 42 to 45. The rate of higher ploidies was 32%. The del(1)(q21), der(13)t(1;?;13)(q11;?;q34), der(11)t(11;?) (q12), del(10)(q22) and 3 other marker chromosomes were common to most cells, and 3 others were found only in some cells. One N11 had the HSR segment from p11 to the distal end. The normal N10, N12, N15, N17 and N19 were absent. Others were either single or paired. There were from 1 to 6 rearranged and unassignable chromosomes. The X chromosome was either single or paired.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1-2 Me-2, 1 PGM1, 1-2 PGM3, 1
Age:	64 years
Gender:	female
Ethnicity:	Caucasian
Comments:	SK-OV-3 cells are resistant to tumor necrosis factor and to several cytotoxic drugs including diphtheria toxin, cis-platinum and adriamycin.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2007 recommended serum:ATCC 30-2020

References:

21869: . Human tumor cells in vitro. New York: Plenum Press; 1975.
22536: Fogh J, et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. 58: 209-214, 1977. PubMed: 833871
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Cell Biology

ATCC® Number: HTB-161™ Order this Item

Price: $279.00

Designations: NIH:OVCAR-3

Depositors: R Ozols, TC Hamilton

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: ovary
Disease: adenocarcinoma
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.

Isolation: **Isolation date:** 1982

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Receptors: androgen receptor, positive; estrogen receptor, positive; progesterone receptor, positive

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11,12
D13S317: 12
D16S539: 12
D5S818: 11,12
D7S820: 10
THO1: 9,9.3
TPOX: 8
vWA: 17

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Cytogenetic Analysis: The cell line is aneuploid human female, with chromosome counts in the sub to near-triploid range. Several normal chromosomes (N11, N13, N14, N15, N16, N17, and N22) are clearly under-represented. Many of these missing chromosomes are represented in the large number of cytogenetically altered chromosomes identified as marker chromosomes. In addition to the marker chromosomes, there are a large number of other structurally abnormal and unassignable chromosomes that are not recognized as markers. Random loss and gain of chromosomes from cell to cell are noted in the exact chromosome counts and in the analysis of the karyotypes.

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

Age: 60 years

Gender: female

Ethnicity: Caucasian

Comments:
The NIH:OVCAR-3 line was established in 1982 by T.C. Hamilton, et al. from the malignant ascites of a patient with progressive adenocarcinoma of the ovary.
Forms colonies in soft agar and has an abnormal karyotype.
Resistant to clinically relevant concentrations of adriamycin, melphalan and cisplatin.
Both cultured cells and xenografts exhibit androgen and estrogen receptors.
Xenograft models have been used to show that treatment with 17 beta estradiol can induce progesterone receptors in this human ovarian carcinoma.
NIH:OVCAR-3 is an appropriate model system in which to study drug resistance in ovarian cancer, and the presence of hormone receptors should be useful for the evaluation of hormonal therapy.

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: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 20%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

Subculturing:
Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
- Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
  Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
- Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting
- Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
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 30-2001
recommended serum:ATCC 30-2020

References:

1127: Hamilton TC, et al. Characterization of a human ovarian carcinoma cell line (NIH:OVCAR-3) with androgen and estrogen receptors. Cancer Res. 43: 5379-5389, 1983. PubMed: 6604576
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Cell Biology

ATCC® Number: CRL-1213™ Order this Item

Price: $429.00

Designations: FR (Rat Dermal Fibroblastic)

Depositors: B Smith

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: Rattus norvegicus (rat)

Morphology: fibroblast

Source:
Organ: skin
Strain: Sprague-Dawley
Disease: normal

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

Age: 18 days gestation

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

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Subculturing:

Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 10 to 20 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.
   Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended
   Medium Renewal: 2 to 3 times per week

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
0.25% (w/v) Trypsin - 0.53mM EDTA in Hank's BSS (w/o Ca++, Mg ++): ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X

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Cell Biology

ATCC® Number:	CCL-226™ Order this Item	**Price:**	$279.00
Designations:	C3H/10T1/2, Clone 8		
Depositors:	C Heidelberger		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Mus musculus* (mouse)		
Morphology:	fibroblast		
Source:	**Strain:** C3H **Organ:** embryo		
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 (Roche FuGENE® Transfection Reagents)		
Tumorigenic:	No		
Antigen Expression:	H-2k		
Cytogenetic Analysis:	Mouse karyotype with a modal number of 80 chromosomes.		
Age:	embryo		

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Comments:

C3H/10T1/2, Clone 8 was isolated by C. Reznikoff, D. Brankow and C. Heidelberger in 1972 from a line of C3H mouse embryo cells. [23019]
The cells are very sensitive to post confluence inhibition of cell division, do not produce tumors in syngeneic mice, have no background of spontaneous transformation, nor do they contain overt endogenous transforming murine leukemia or sarcoma viruses. [22697]
The cells are contact sensitive.
There is no detectable background spontaneous transformation.
They are highly susceptible to transformation by chemical agents. [1208]
Tested and found negative for ectromelia virus (mousepox).
NOTE: THE INOCULATION DENSITY, FEEDING AND HARVESTING SCHEDULES MUST BE FOLLOWED RIGIDLY IF THE LINE IS TO RETAIN ITS ESSENTIAL CHARACTERISTICS.
THE BATCH OF SERUM USED FOR GROWTH AND FOR TRANSFORMATION ASSAYS MAY AFFECT BOTH THE MORPHOLOGY OF THIS LINE AND THE RESULTS OBTAINED.
Monolayers established and maintained for the standard transformation assay should be free of all foci after 6 weeks. [1208]
The donor recommends that the line be used between the 5th and 15th passages only.

Propagation:

ATCC complete growth medium: The base medium for this cell line is Eagle's Basal medium with 2 mM L-glutamine , 1.5 g/L sodium bicarbonate and Earle's BSS. To make the complete growth medium, add the following components to the base medium: heat-inactivated fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%

Subculturing:

Protocol: Remove medium, and rinse with 0.25% trypsin, 0.53 mM 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. SUBCULTURE MUST BE DONE BEFORE THE CULTURE REACHES CONFLUENCE.
Subcultivation Ratio: Seed new flasks at 2000 viable cells/sq cm.
Medium Renewal: Once between subcultures if necessary

Preservation:

Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor temperature

References:

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Cell Biology

ATCC® Number: TIB-71™ Order this Item

Price: $279.00

Designations: RAW 264.7

Depositors: WC Raschke

Biosafety Level: 2

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: monocyte/macrophage

Source:
Tissue: ascites
Strain: BALB/c
Disease: Abelson murine leukemia virus-induced tumor
Cell Type: macrophage; Abelson murine leukemia virus transformed

Cellular Products: lysozyme [1207]

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: Biological response [92560]
transfection host (Roche FuGENE® Transfection Reagents)

Receptors: complement (C3) [1207]

Antigen Expression: H-2d

Age: adult

Gender: male

Comments: This line was established from a tumor induced by Abelson murine leukemia virus. They are negative for surface immunoglobulin (sIg-), Ia (Ia-) and Thy-1.2 (Thy-1.2)This line does not secrete detectable virus particles and is negative in the XC plaque formation assay. The cells will pinocytose neutral red and will phagocytose latex beads and zymosan. They are capable of antibody dependent lysis of sheep erythrocytes and tumor cell targets. LPS or PPD treatment for 2 days stimulates lysis of erythrocytes but not tumor cell targets.Data communicated in Feb. 2007 by Dr Janet W. Hartley, indicates the expression of infectious ecotropic MuLV closely related, if not identical, to the Moloney MuLV helper virus used in the original virus inoculum. The cells also express polytropic MuLV, unsurprisingly based on the mouse passage history of the virus stocks [PubMed 18177500].

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Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Subcultures are prepared by scraping. For a 75 cm2 flask, remove all but 10 ml culture medium (adjust amount accordingly for other culture vessels). Dislodge cells from the flask substrate with a cell scraper; aspirate and add appropriate allquots of the cell suspension into new culture vessels. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Replace or add medium every 2 to 3 days.
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020
References:	1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031 1207: Raschke WC, et al. Functional macrophage cell lines transformed by Abelson leukemia virus. Cell 15: 261-267, 1978. PubMed: 212198 32443: Denlinger LC, et al. Regulation of inducible nitric oxide synthase expression by macrophage purinoreceptors and calcium. J. Biol. Chem. 271: 337-342, 1996. PubMed: 8550583 32466: Hambleton J, et al. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad. Sci. USA 93: 2774-2778, 1996. PubMed: 8610116 32553: Taylor GA, et al. Identification of anovel GTPase, the inducibly expresed GTPase, that accumulates in response to interferon gamma. J. Biol. Chem. 271: 20399-20405, 1996. PubMed: 8702776 32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: 8855306 33046: Panneerselvam K, Freeze HH. Mannose enters mammalian cells using a specific transporter that is insensitive to glucose. J. Biol. Chem. 271: 9417-9421, 1996. PubMed: 8621609 33076: Lokuta MA, et al. Mechanisms of murine RANTES chemokine gene induction by newcatle disease virus. J. Biol. Chem. 271: 13731-13738, 1996. PubMed: 8662857 33162: Taylor MF, et al. In vitro efficacy of morpholino-modified antisense oligomers directed against tumor necrosis factor-alpha mRNA. J. Biol. Chem. 271: 17445-17452, 1996. PubMed: 8663413 92560: Standard Practice for Testing for Biological Responses to Particles in Vitro. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 1903-98R03, 16173094: Hartley JW, et al. Expression of infectious murine leukemia viruses by RAW264.7 cells, a potential complication for studies with a widely used mouse macrophage cell line. Retrovirology. 4: 5:1, 2008. PubMed 18177500.

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Cell Biology

ATCC® Number: CRL-2192™ Order this Item

Price: $279.00

Designations: NR8383 [AgC11x3A, NR8383.1]

Depositors: RJ Helmke

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: mixed, adherent and suspension

Organism: Rattus norvegicus (rat)

Morphology: macrophage

Source:
Strain: Sprague-Dawley
Organ: lung
Disease: normal
Cell Type: macrophage (alveolar);

Cellular Products: transforming growth factor beta (TGF beta); interleukin 1 (IL-1); interleukin 6 (IL-6)

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:** August 3, 1983

Receptors: Fc

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Comments:

NR8383 (normal rat, August 3, 1983) was established from normal rat alveolar macrophage cells obtained by lung lavage.
The cells were cultured in the presence of gerbil lung cell conditioned medium for approximately 8 to 9 months.
Subsequently the requirement for exogenous growth factors was lost.
NR8383 cells were cloned and subcloned from single cells by limiting dilution, and then subcloned from soft agar three times.
The cells exhibit characteristics of macrophage cells:
Phagocytosis of zymosan and Pseudomonas aeruginosa, nonspecific esterase activity, Fc receptors, oxidative burst,IL-1, TNF beta and IL-6 secretion, and replicative response to exogenous growth factors.
The cells respond to appropriate microbial, particulate or soluble stimuli with phagocytosis and killing.
NR8383 cells respond to bleomycin by secreting latent transforming growth factor (TGF beta).
Stimulation with bleomycin also increases TGF beta mRNA expression.
These cells are sensitive to endotoxin.
LPS levels of 1 to 10 ng/ml inhibit replication by 50%.
LPS inhibition is nontoxic and reversible even after levels up to 0.001 mg/ml for extended periods.
The NR8383 cell line provides a homogenous source of highly responsive alveolar macarophages which can be used in vitro to study macrophage related activities.

Propagation:

ATCC complete growth medium: Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 85%; heat inactivated fetal bovine serum, 15%
Temperature: 37.0°C

Subculturing:

Protocol: Cultures can be maintained by transferring floating cells to additional flasks. Adherent cells may be harvested by scraping. Upon reseeding, about one half of the cells will re-attach. Cultures are most successful when set up at a floating cell concentration of 1 to 4 X 10 exp5 viable cells/ml.
Medium Renewal: Two to three times weekly

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004
purified RNA:ATCC CRL-2192R

References:

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23566: Henderson SA, et al. Nitric oxide reduces early growth response-1 gene expression in rat lung macrophages treated with interferon-gamma and lipopolysaccharide. J. Biol. Chem. 269: 25239-25242, 1994. PubMed: 7523382
36466: Huang S, et al. Rat KC cDNA cloning and mRNA expression in lung macrophages and fibroblasts. Biochem. Biophys. Res. Commun. 184: 922-929, 1992. PubMed: 1374243

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Cell Biology

ATCC® Number: CRL-2808™ Order this Item

Price: $338.00

Designations: WM1552C [Part of the Wistar Special Collection]

Depositors: M Herlyn

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: Spindle-shaped

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Source: **Organ:** skin
Tumor Stage: stage 3?
Disease: primary superficial spreading melanoma (SSM) (radial growth phase (RGP)/vertical growth phase (VGP))

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

Restrictions: Distribution of this cell line is governed by the Wistar Special Collection Material Transfer Agreement.

Isolation: **Isolation date:** February 8, 1988

Tumorigenic: Yes

Age: 72 years

Gender: male

Comments: The WM1552C line was established from a primary superficial spreading melanoma (SSM) in radial growth phase (RGP)/vertical growth phase (VGP) from the buttocks of a patient on 02/08/88.

Propagation: **ATCC complete growth medium:** 2% Tumor Medium (Tu2%) containing a 4:1 mixture of MCDB 153 medium with 1.5 g/L sodium bicarbonate and Leibovitz's L-15 medium with 2 mM L-glutamine supplemented with 0.005 mg/ml bovine insulin, 1.68 mM CaCl2, and 2% fetal bovine serum.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/cm2 is recommended. .
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 1 X 10(4) and 7 X 10(4) cells/cm2
Subcultivation Ratio: A subcultivation of 1:3 to 1:4 is recommended
Medium Renewal: Two to three times weekly

Preservation:

Freeze medium: Complete growth medium supplemented with an additional 8% fetal bovine serum and 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 42 hours

Related Products: recommended serum:ATCC 30-2020

References:

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89403: Satyamoorthy K, et al. Melanoma cell lines from different stages of progression and their biological and molecular analyses. Melanoma Res. 7: S35-S42, 1997. PubMed: 9578415

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