Modification Form for Permit BIO-LRCC-0021

Permit Holder: Alison Allan

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

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

David Goodale

Alysha Croker

~~Irene Ma~~

Jenny Chu

Lori Lowes

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	E.coli NEB 5a	
Approved Primary and Established Cells	Established: [Human] Breast Cancer -21NT, 21PT, MC7, MDA-MB-231, MDA-MB-435S, MDA-MB-468, SUM 149PT, SUM 159PT, SUM 1315MO2, T47D, MCF 10A, SKBR3, Prostate Cancer - VCap, LNCap. [Rodent] -	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmids]: pcDNA 3.1 (+). [Gene transfected]: Human ALDH1a1, Human OPN	Lentivirus
Approved Use of Animals	mouse	
Approved Biological Toxin(s)	cholera	

Approved Gene Therapy		
Approved Plants and Insects		

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

Signature of Permit Holder: March 30, 2012.

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

Date of Last Biohazardous Agents Registry Form: Sep 14, 2011

Date of Last Modification (if applicable):

BioSafety Officer(s)*: Dave Ryder APRIL 10, 2012

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

Chair, Biohazards Subcommittee: **Date:**

------- Original Message --------
Subject: Fwd: Re: BIO-LRCC-0021 MOD - ALLAN
Date: Fri, 25 May 2012 15:05:35 -0400
From: David Goodale <David.Goodale@lhsc.on.ca>
To: Alison Allan <Alison.Allan@lhsc.on.ca>, Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

New Info

Here are the answers to the questions.

The Committee requires more detail on the work being done with the Lentivirus such as which genes are targeted for knockdown and the effect that this will have on the cells.

We hypothesize that primary tumors with a mesenchymal phenotype will generate more CTCs, and lead to more metastases, then primary tumors with an epithelial phenotype. However, CTCs generated by mesenchymal primary tumors will be less likely to be detectable by the CellSearch system then those generated by epithelial primary tumors. In order to test this hypothesis we will be inducing a mesenchymal phenotype in an epithelial cell line by down-regulating expression of the E-cadherin gene.We will also be inducing an epithelial or more differentiated phenotype in a mesenchymal cell line by down-regulating expression of the stem cell genes. Aldehyde dehydrogenase (ALDH) and CD44. We will then compare the behavioural changes of these cells to those of their parent cells, using both in vitro assays in tissue culture and in vivo metastasis assays in mice. This work will help us to better understand the biology underlying these rare cells and ultimately aid in their interpretation in the clinic.We expect that down-regulation of E-cadherin will result in enhancedmigration of cells, whereas down-regulation of ALDH and CD44 will result in reduced migration of cells.

(also attached updated BARF for page 3 of 10)

Clarification is also required regarding the containment level of the work as the form refers to both Level 2 and Level 2 plus.

Work with Level 2+ agents (such as Lentivirus) is conducted in Rm A4-822. All other level 2 work (suchs as human blood) are done in level 2 BSC's.

What containment level is the mouse work done at?

Mouse work will be done in a Level 2+ facility.

Thanks,
David

DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

Biological Agents: human cancer cell lines (MDA-MB-468 and MDA-MB-468 LN), Lentiviral Vector System from OPEN BioSystems, Bacteria

Usage: Cells are maintained under routine cell culture techniques (Grown in CO2 incubators, passaged and handled in a laminar flow BCS. Cells are used in-vitro for 2Dand 3D assays (ie proliferation. migration assay).

Cells for in-vivo mouse studies will be injected (mammary fat pad) for Observation of tumour development and metastisis. Mice will be handled and kept at the VRL vivarium which is a level II animal-handling facility.

The Lentiviral Vector System will be used to transfect a shRNA library into cancer cells. The lentivirus contains deletion in the LTR region which prevents transcription of the virus in transfected cell lines. The lentivirus is made by transfecting three different plasmids into cells that will make lentivirus particles. The cells typically used are called MDA-MB-468 cells. After 24-72 hrs post-transfection, media is collected which contains viral particles released by the by the MDA-MD-468 cells. This media, usually 2-3ml is immediately to another well which contains the cells that will be infected with virus. After 4 days of infection, this media is removed and bleached. No vacuum aspiration is used, only a disposable 10ml pipette is used and is bleached as well.
All viral transfection work will be performed only in room LRCP A4-822 which is designated as Level 2+ for viral work.

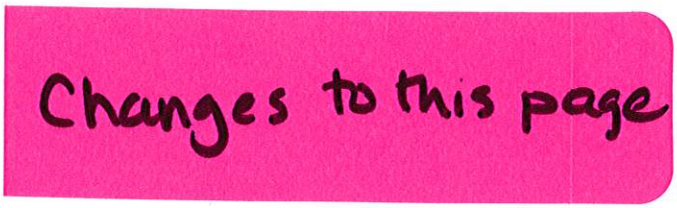
Bacterial strains, containing recombianant protein expression plasmid or vector DNA, Are used in the mass production of protein or in the amplification of plasmid DNA.

Storage: Frozen at -20, -80 and -150

Disposal: All items used in viral work are bleached prior to disposal. Liquids are either autoclaved or bleached prior to being deposited into the sewer. Plasticware that has been in contact with biological agent(s) are placed in yellow biohazard bags inside cardboard boxes. Disposable glassware is placed in yellow biohazard plastic pails. These biohazard boxes and pails are taken away by Steicycle (licensed waste carrier) who then autoclave the items prior to disposal in landfill.

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

The majority of deaths caused by breast cancer do not occur as a result of the primary breast tumor, but rather from the spread of cancer cells to other vital organs. This process is known as metastasis, and the tumors formed in other organs are called metastases. Breast cancer cells use the bloodstream to travel through the body and these cells are called circulating tumor cells (CTCs). CTCs are very rare and require sensitive equipment to detect and count them accurately. Our lab has 1 of only 3 clinical instruments (called the CellSearch system) in Canada capable of doing this. Although the CellSearch system is currently the gold standard in CTC enumeration it has limitations with detecting a subset of non-epithelial or stem-like cells CTCs (mesenchymal phenotype). It has been suggested that these mesenchymal cells are a marker of poor prognosis due to their increased aggressiveness. We hypothesize that primary tumors with a mesenchymal phenotype will generate more CTCs, and lead to more metastases, then primary tumors with an epithelial phenotype. However, CTCs generated by mesenchymal primary tumors will be less likely to be detectable by the CellSearch system then those generated by epithelial primary tumors. In order to test this hypothesis we will be inducing a mesenchymal phenotype in an epithelial cell line by down-regulating expression of the E-cadherin gene.We will also be inducing an epithelial or more differentiated phenotype in a mesenchymal cell line by down-regulating expression of the stem cell genes. Aldehyde dehydrogenase (ALDH) and CD44. We will then compare the behavioural changes of these cells to those of their parent cells, using both in vitro assays in tissue culture and in vivo metastasis assays in mice. This work will help us to better understand the biology underlying these rare cells and ultimately aid in their interpretation in the clinic.We expect that down-regulation of E-cadherin will result in enhancedmigration of cells, whereas down-regulation of ALDH and CD44 will result in reduced migration of cells.



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?
DH5aplha	**see attached list**	**see attached list**	**see attached list**	**no**	**no**	**amplification of plamid DNA**

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Lentivirus	**pGIPZmir library**	**Open Biosytems**	**complete genome library**	**decrease in gene expression**

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

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

Additional Comments: **Viral work (level 2+) is only conducted in Room A4-822. No viral work done in Rms A4-114, A4-909 whcich are classified as Level 2.**

7.0 Animal Experiments

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

7.2 Name of animal species to be used **Mouse**

7.3 AUS protocol **# 2009-064, 2009-062**

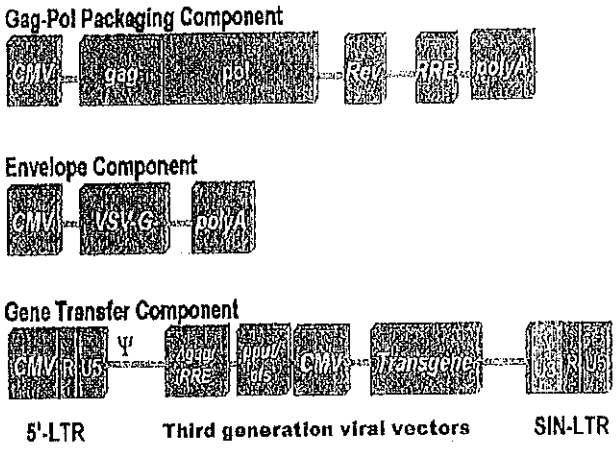
7.4 List the location(s) for the animal experimentation and housing. **Victoria Research Labs Vivarium**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☒ YES, specify: **srable transfected cancer cell lines injected into mice**

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: **cells injected into mammary fat pad for tumour formation and do not expect cells to be cleared**

OPEN BIOSYSTEMS

Safety and handling of the shRNAmir Lentiviral Vector System



Typical 'third generation' lentiviral vectors provide a three part packaging system that does have the unwanted potential for production of replication competent retrovirus. These lentiviral vector stocks have been shown to recombine during reverse transcription in the targeted cells. This will re-join the viral protein-coding sequences of the packaging construct and *cis*-acting sequences of the vector generating *env*-minus recombinants (LTR-*gag-pol*-LTR). Mobilization of recombinant lentiviral genomes was also demonstrated but was dependent on pseudotyping of the vector core with an exogenous envelope protein. 5′ sequence analysis has demonstrated that recombinants consist of U3, R, U5, and the Ψ packaging signal joined with an open *gag* coding region. Analysis of the 3 end has mapped the point of vector recombination to the poly (A) tract of the packaging construct's mRNA.[4]

The Trans-Lentiviral™ packaging system is designed with the latest and best safety technologies of any expression system. The trans-lentiviral packaging vectors, a new class of HIV-based vectors, enhance the third-generation system by splitting the *gag-pol* component of the packaging construct into two parts. The first expresses *Gag/Gag-Pro*. The second expresses *Pol* (RT and IN) fused with *Vpr*. Unlike other lentiviral vectors, the trans-lentiviral vectors do not form recombinants capable of DNA mobilization. These results indicate the Trans-vector™ design prevents the generation of *env*-minus recombinant lentivirus containing a functional *gag-pol* structure (LTR-*gag-pol*-LTR), which is absolutely required for retroviral DNA mobilization.

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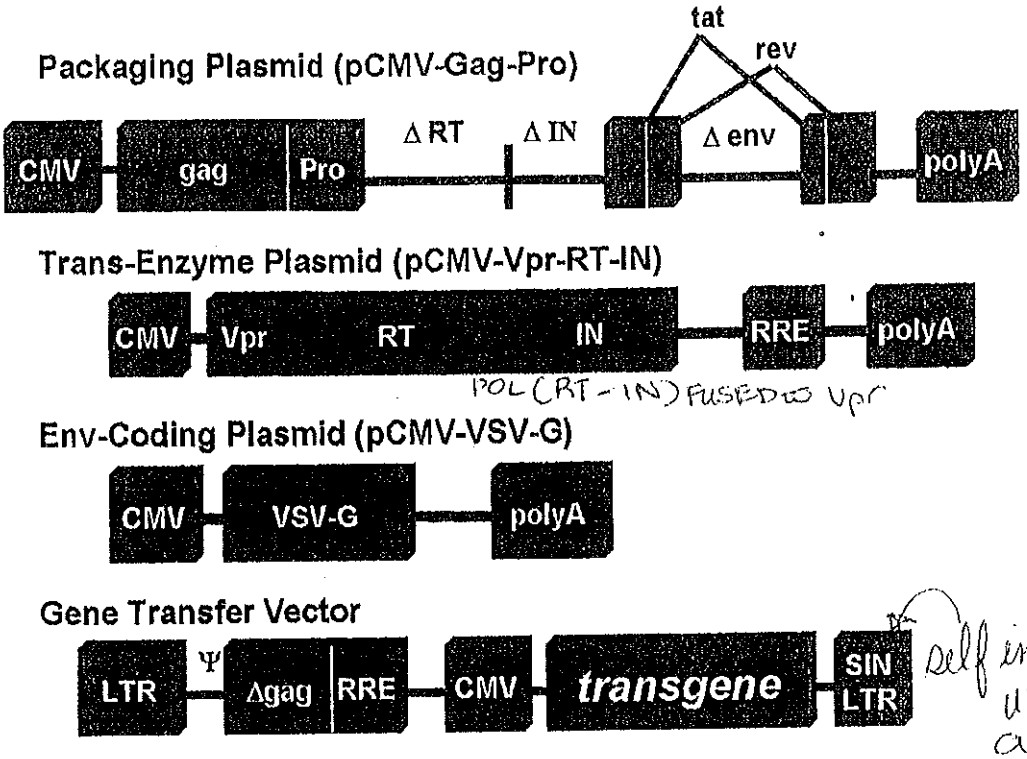
Trans-Lentiviral™ packaging system components



The Expression Arrest™ shRNAmir lentiviral vectors are **self inactivating (sin)** expression vectors. The viral vectors contain a specifically designed long terminal repeat (LTR) derived from the native virus. These LTR's differs from the native by several point mutations and a deletion, enhancing transcriptional activation and decreasing transcriptional suppression in embryonic stem and embryonal carcinoma cells.

Self inactivating vectors are constructed by deleting the enhancer and/or the promoter in the U3 region of the 3' LTR. During reverse transcription, a circular intermediate is formed that transfers the deletion to the 5' LTR of the pro-viral DNA. The deletion abolishes any transcriptional activity driven by the LTR so that no full-length vector RNA is produced in transduced cells. Following a single round of replication, the changes are copied into both 5' and 3' LTRs resulting in inactive provirus. However, any promoter internal to the LTRs in such vectors will still be active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes.

The Expression Arrest™ shRNAmir lentiviral vectors are infectious **only** when packaged in a cell line with appropriate tropism but is not replication competent. Virus produced by both transient and stable transfections can infect target cells and transmit target genes; however, it cannot replicate within target cells because the viral structural genes are absent.

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Viral packaging

To effectively package lentivirus, the viral gag, pol and env genes- necessary for particle formation and replication- are co-transfected into the packaging cell line. The separate introduction of the structural genes minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. Viral expression vectors provide the packaging signal, transcription and processing elements, and a target gene or shRNA. Simultaneous transfection of the gene transfer vector and viral packaging plasmids into a packaging cell line produces high-titer, replication-incompetent virus.

The protocols for viral cell packaging require the producing, handling and storing of infectious lentivirus. An understanding of safe laboratory practices and potential viral hazards is necessary. Appropriate NIH, regional, and institutional guidelines apply, as well as specific guidelines for other countries. In the United States, NIH guidelines require that viral production and transduction be performed in a Biosafety Level 2 (BL2) facility for more information about BL2 guidelines the section below is provided (**http://bmbl.od.nih.gov/contents.htm**)

Excerpt from the NIH Recommendations on Biosafety in Biomedical and Microbiological Laboratories

Biosafety Level 2 (BSL-2) is similar to Biosafety Level 1 (BSL-1) and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the

investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
    a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.
    b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be

carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needle less systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

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D. Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

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Handle all lentiviruses in compliance with established institutional guidelines. Since safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution prior to use of the trans-lentiviral™ packaging system.

Note: Viral supernatants produced using leniviral packaging plasmids, depending on your shRNA insert, may contain potentially hazardous recombinant virus. All users must exercise caution in the production, use and storage of recombinant virus, especially those with amphotropic or dualtropic host ranges.

OPEN BIOSYSTEMS

References

1. Chen W, Wu X, Levasseur DN, Liu H, Lai L, Kappes JC, Townes TM.
Lentiviral vector transduction of hematopoletic stem cells that mediate long-term reconstitution of lethally irradiated mice.
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2. Coffin, JM, Hughes SH and Varmus H. Eds.
Retroviruses
1997 Cold Spring Harbor Laboratory Press.

3. Kappes JC, Wu X, Wakefield JK.
Production of trans-lentiviral vector with predictable safety.
Methods Mol Med. 2003;76:449-65.
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4. Kappes JC, Wu X.
Safety considerations in vector development.
Somat Cell Mol Genet. 2001 Nov;26(1-6):147-58.
PMID: 12465466

5. Wu X, Wakefield JK, Liu H, Kappes JC.
Analysis of lenti- and trans-lentiviral vector genetic recombination.
Dev Biol (Basel). 2001;106:237-48; discussion 249, 253-63.
PMID: 11761237

6. Wu X, Wakefield JK, Liu H, Xiao H, Kralovics R, Prchal JT, Kappes JC.
Development of a novel trans-lentiviral vector that affords predictable safety.
Mol Ther. 2000 Jul;2(1):47-55.
PMID: 10899827

Expression Arrest™ GIPZ lentiviral shRNAmir-Glycerol Stocks

APPLICABLE CATALOG NUMBERS

Catalog Numbers	Description
RHS4430	Human GIPZ lentiviral shRNAmir individual clone
RMM4431	Mouse GIPZ lentiviral shRNAmir individual clone
RHS4346	Non-silencing-GIPZ lentiviral shRNAmir control-Glycerol stock
RHS4349	pGIPZ lentiviral empty vector - Glycerol stock
RHS4371	GAPDH-GIPZ lentiviral shRNAmir positive control-Glycerol stock
RHS4480	EG5-GIPZ lentiviral shRNAmir positive control-Glycerol stock
RHS4477	Human GIPZ lentiviral shRNAmir library subscription
RMM4501	Mouse GIPZ lentiviral shRNAmir library subscription

PRODUCT DESCRIPTION

The GIPZ lentiviral shRNAmir library was developed by Open Biosystems in collaboration with Dr. Greg Hannon (CSHL) and Dr. Steve Elledge (Harvard). This library combines the design advantages of microRNA-adapted shRNA (shRNAmir) with the pGIPZ lentiviral vector to create a powerful RNAi trigger capable of producing RNAi in most cell types including primary and non-dividing cells.

QUALITY CONTROL

Open Biosystems checks all cultures for growth prior to shipment.

SHIPPING AND STORAGE

Individual constructs are shipped as bacterial cultures of *E. coli* (Prime Plus) in LB-Lennox (low salt) broth with 8% glycerol, 100µg/ml carbenicillin and 25µg/ml zeocin. Individual constructs are shipped on wet ice. Collections are shipped in 96 well plate format on dry ice. Individual constructs and collections should be stored at -80°C.

TO ALLOW ANY CO_2 THAT MAY HAVE DISSOLVED INTO THE MEDIA FROM THE DRY ICE IN SHIPPING TO DISSIPATE, PLEASE STORE CONSTRUCTS AT –80°C FOR AT LEAST 48 HOURS BEFORE THAWING.

Important Safety Note:
Follow NIH guidelines regarding lentiviral production and transduction; follow Biosafety Level 2 (BL2) or BL2+ laboratory criteria.
NIH Agent Summary Statement: http://bmbl.od.nih.gov/viral2.htm#retro
NIH Biosafety Level 2 Description: http://bmbl.od.nih.gov/sect3bsl2.htm
NIH/RAC "Guidance on Biosafety Considerations for Research with Lentiviral Vectors":
http://www4.od.nih.gov/oba/RAC/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

Please note that GIPZ vectors are not compatible with third generation packaging systems such as ViraPower from Invitrogen. We recommend the TransLentiviral Packaging system for use with our vectors.

PRODUCT INFORMATION

The product manual for the Expression Arrest pGIPZ lentiviral shRNAmir collection is available for download using the following link:
https://www.openbiosystems.com/collateral/rnai/pi/pGIPZ-manual.pdf

ThermoFisher
SCIENTIFIC

Material Safety Data Sheet

Creation Date 18-Sep-2009 Revision Date 24-May-2010 Revision Number 2

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Viral Particles - including GIPZ, Lenti-ORF, and shMIMIC

Cat No. VGHXXXX, VGMXXXX, VGRXXXX, RHS4348, RHS4372, RHS4351, HMRXXXX, VSHXXXX (Excluding Arrayed Libraries), OHSXXXX

Synonyms No information available.

Recommended Use For research use only

Company
Thermo Fisher Scientific
Open Biosystem Products
601 Genone Way # 2100
Huntsville, AL 35806 United States
Tel: (303) 604-9499
Fax:(303) 604-9680

Emergency Telephone Number
Chemtrec US: (800) 424-9300
Chemtrec EU: (202) 483-7616

2. HAZARDS IDENTIFICATION

WARNING!

Emergency Overview

Potential Biohazard. Handle in accordance with good industrial hygiene and safety practice. May cause eye, skin, and respiratory tract irritation . Shipped on dry ice.

Appearance Yellow **Physical State** Liquid **odor** No information available

Target Organs None known.

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	May cause irritation
Skin	May cause irritation
Inhalation	May cause irritation of respiratory tract
Ingestion	Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea

Chronic Effects None known.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
DMEM	NA	1 - 99
Viral Particles	NA	1 - 99

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire..

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
None known.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA	Health 1	Flammability 0	Instability 0	Physical hazards N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions	Use personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing.
Environmental Precautions	Should not be released into the environment.
Methods for Containment and Clean Up	Soak up with inert absorbent material. Keep in suitable and closed containers for disposal.

7. HANDLING AND STORAGE

Handling	Handle in accordance with good industrial hygiene and safety practice. Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation. This material should be handled at the biosafety level 2 (BSL2) as required by OSHA Bloodborne Pathogen Rule (29 CFR 1910.1030.7).
Storage	Keep container tightly closed. Keep at -80°C.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures	Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.
Exposure Guidelines	This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

NIOSH IDLH: Immediately Dangerous to Life or Health

Personal Protective Equipment

Eye/face Protection	Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166
Skin and body protection	Wear appropriate protective gloves and clothing to prevent skin exposure
Respiratory Protection	Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Yellow
odor	No information available
Odor Threshold	No information available.
pH	Not applicable
Vapor Pressure	No information available.
Vapor Density	No information available.

9. PHYSICAL AND CHEMICAL PROPERTIES

Viscosity	No information available.
Boiling Point/Range	Not applicable
Melting Point/Range	No information available.
Decomposition temperature	No information available.
Flash Point	Not applicable
Evaporation Rate	No information available.
Specific Gravity	No information available.
Solubility	No information available.
log Pow	No data available

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Conditions to Avoid	Excess heat.
Incompatible Materials	None known
Hazardous Decomposition Products	None known
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions .	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information	No acute toxicity information is available for this product
Component Information	
Irritation	No information available.
Toxicologically Synergistic Products	No information available.

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product
Sensitization	No information available.
Mutagenic Effects	No information available.
Reproductive Effects	No information available.
Developmental Effects	No information available.

Teratogenicity	No information available.
Other Adverse Effects	The toxicological properties have not been fully investigated..
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Do not empty into drains.

Persistence and Degradability	No information available
Bioaccumulation/ Accumulation	No information available
Mobility	No information available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods	Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

TDG

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IATA

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IMDG/IMO

14. TRANSPORT INFORMATION

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

15. REGULATORY INFORMATION

International Inventories

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
Not Applicable

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know
Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
D3 Biohazardous infectious materials

16. OTHER INFORMATION

Prepared By	Regulatory Affairs Thermo Fisher Scientific Tel: (412) 490-8929
Creation Date	18-Sep-2009
Print Date	24-May-2010
Revision Summary	"****", and red text indicates revision

Disclaimer

The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	Price:	**$264.00**
Designations:	MDA-MB-468	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

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

Related Cell Culture Products

Isolation:
Isolation date: 1977

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

Receptors:
epidermal growth factor (EGF)
transforming growth factor alpha (TGF alpha)

Tumorigenic:
Yes

Antigen Expression:
Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)

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

Cytogenetic Analysis:
modal number = 64; range = 60 to 67.
The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.; A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes.; Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, A
GLO-I, 1-2
Me-2, 1-2

	PGM1, 1 PGM3, 2
Age:	51 years
Gender:	female
Ethnicity:	Black
Comments:	The MDA-MB-468 cell line was isolated in 1977 by R. Cailleau, et al., from a pleural effusion of a 51-year-old Black female patient with metastatic adenocarcinoma of the breast. Although the tissue donor was heterozygous for the G6PD alleles, the cell line consistently showed only the G6PD A phenotype.There is a G -> A mutation in codon 273 of the p53 gene resulting in an Arg -> His substitution.EGF receptor is present at 1 X 10(6) per cell.
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.R emove and discard culture medium. 2.Br iefly 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 appr opriate aliquots of the cell suspension to new culture vessels. 6.I ncubate cultures at 37°C. **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:	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 recommended serum:ATCC 30-2020 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008
	1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337 22429: Siciliano MJ, et al. Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker. Cancer Res. 39: 919-922,

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 Alison Allan
DEPARTMENT	Oncology
ADDRESS	790 Commissioners Rd East
PHONE NUMBER	519-685-8600 ext 55134
EMERGENCY PHONE NUMBER(S)	(519) 852-9176
EMAIL	Alison.allan@lhsc.on.ca

Location of experimental work to be carried out: Building(s): LRCP, VRL 4th floor; Room(s) A4-114 & A4-909

*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: OICR, CIHR, CFI, Province of Ontario (ERA)
GRANT TITLE(S):
1. Role of ALDH+/CD44+ stem-like cells in breast cancer progression and treatment
2. Circulating tumor cell (CTC) analysis and characterization using novel microfiltration technology and FISH: A correlative biology companion study to the NCIC-CTG Phase II clinical trial IND.205
3. Understanding breast cancer metastasis
4. A laboratory for the investigation of cellular and molecular mechanisms of breast cancer metastasis

List all personnel working under Principal Investigator's supervision in this location:

Name	E-mail Address	Date of Biosafety Training
David Goodale	david.goodale@lhsc.on.ca	May 2006
Alysha Croker	acroker@uwo.ca	September 2006
Lori Lowes	llowes@uwo.ca	September 2008
Jenny Chu	jchu87@uwo.ca	September 2010
Irene Ma	ima@uwo.ca	September 2009
Ying Xia	ying.xia@lhsc.on.ca	September 2007

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.

We will be using established, immortalized human cancer cell lines. When the cells are not in culture they will be frozen and stored at either -80°C or -140°C/liquid nitrogen. After the cells are frozen and if any are still growing, they will be bleached out if no longer needed.

We will also be injecting the cells in to mice for experiments as outlined in our approved animal protocols 2008-028, 2009-062 and 2009-064. We also use chemotherapy agents (paclitaxel, doxirubicin and/or cyclophosphamide). All mice will be handled with PPE by animal staff and laboratory staff that will be working with them. Cages will be labeled with cytotoxic stickers to ID them. After the mouse experiments are done the carcasses will be incinerated.

We will need to use cholera toxin to grow one cell line (MCF-10A – see cell info sheet) This cell line requires this toxin in order to grow. We will only order cholera toxin when these cells are growing and keep a limited supply of this item within the lab. It will be disposed of in 10% bleach as it is only in the media required by the cells.

We will be receiving human blood (from cancer patients and normal donors) from the phlebotomy and core labs at LRCP/LHSC Victoria campus to analyze on an instrument called the CellSearch for various clinical and translational research projects. The blood with be handled with PPE and once we are finished with it any remainder will be bleached. The CellSearch instrument has a built-in system to remove and dispose of human blood after it has been used and this instrument has been approved by Health Canada and the US FDA.

Please include a one page research summary or teaching protocol.

Solid cancers such as breast and prostate cancer are leading causes of death in Canada, due mainly to the propensity of these tumors to metastasize to distant organs. To understand and study how these cancer cells behave within the body, we use in vitro and in vivo pre-clinical model systems encompassing cultured human cancer cells, patient blood samples, and mouse models of metastasis.

We currently have several projects that are studying different aspects of human cancer metastasis. These projects involve the study of cellular and molecular determinants of metastasis, including circulating tumor cells (CTCs) and cancer stem cells (CSCs).

For the CSC studies, we trying to understand how these cells behave within the body to contribute to metastasis, as well as how to best treat and/or prevent metastasis by targeting these cells. For the CTC studies, we are working with several clinicians at the LRCP to look for CTCs in patient blood samples. We have a specialized instrument called the CellSearch System that is able to capture and characterize these rare CTCs. These projects include studies in breast cancer, prostate cancer, colorectal cancer, esophageal cancer, and head & neck cancer.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES **X 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 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 NEB 5a	O Yes O No	O Yes O No	O Yes O No		Biolabs	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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? **XYES** 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 No**		Not applicable
Rodent	O Yes **X No**		
Non-human primate	O Yes **X No**		
Other (specify)	O Yes **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	**X Yes** O No	See attached sheet	1	ATCC, Asterand
Rodent	**X Yes** O No	4T1	1	ATCC
Non-human primate	O Yes **X No**			
Other (specify)	O Yes **X No**			

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

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required **X 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? **X 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	LRCP/LHSC Labs	O Yes **X Unknown**		O 1 **X 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)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		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? **X YES** O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O 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
NEB 5a competent E.coli	*pcDNA 3.1(+)*	*Invitrogen*	*Human ALDH1a1*	*Increased metastatic capacity of cells in vitro*
NEB 5a competent E.coli	*pcDNA 3.1(+)*	*Invitrogen*	*Human OPN*	*Increased metastatic capacity of cells in vitro and in vivo*

* 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 **X 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 _____ **X NO**
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ **X NO**
- SV 40 Large T antigen O YES **X NO**
- E1A oncogene O YES **X NO**
- Known oncogenes O YES, please specify _____ **X NO**
- Other human or animal pathogen and or their toxins O YES, please specify _____ **X 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? YES **X 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? **X YES** NO If no, please proceed to section 7.0

6.2 Name of animal species to be used: Mouse

6.3 AUS protocol #s: 2008-028, 2009-062, 2009-064

6.4 Will any of the agents listed in section 4.0 be used in live animals **XYES**, specify: both listed

6.5 Will the agent(s) be shed by the animal: O YES **X NO**, please justify:
Once the transfected cancer cells are placed in the animal, the cells are not shed outside the animal due to the fact the cells cannot survive outside 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 **X 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? **X YES** O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s): ~~Chlorea~~ Toxin
cholera JS

8.3 Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used. JS

8.3 What is the LD_{50} (specify species) of the toxin: 250ul/kg → 250 ug/kg (assume density = 1g/mL

8.4 How much of the toxin is handled at one time*? 50 ug

8.5 How much of the toxin is stored*? 0.5 mg

8.6 Will any biological toxins be used in live animals? O YES, Please provide details: **X 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 **X 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 **X 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 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 X 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
X **YES**, date of most recent biosafety inspection: December 10, 2010
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): R-06-000599 (see attached)
Bio-LRCC-0021

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.
PPE will be used for staff such as gloves, labcoat, safety glasses, and a biological hood.

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:

Area will be washed with warm water and soap ASAP and if needed the person will seek medical attention through Occupational Health & Safety, their family doctor, or the ER.

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: 17 August 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: Sept 14 2011

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Sept 9, 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: Aug. 24, 2011

Approval Number: Bio-LRCC-0021 Expiry Date (3 years from Approval): Sep 13, 2014

Special Conditions of Approval: . The AUS office will contact the lab regarding the testing of cell lines for pathogens.

NEW ENGLAND BioLabs Inc.
New England Biolabs
240 County Road
Ipswich, MA 01938

Telephone: (978) 927-5054
Toll free: (800) 632-5227
Fax: (978) 921-1350
Email: info@neb.com
Revision Date: 9/10

MATERIAL SAFETY DATA SHEET (MSDS)

NEB #C2523

SECTION 1–CHEMICAL INFORMATION

Product Name: NEB Express Competent *E. coli* (High Efficiency) Cas.# None

SECTION 2–COMPOSITION/INFORMATION ON INGREDIENT

1.	Glycerol	1–10%	Cas.# 56-81-5
2.	Dimethyl Sulfoxide	1–10%	Cas.# 67-68-5

The ingredients listed in this section include only those items that have more than 1% of a component classified as hazardous and 0.1% of a component classified as carcinogenic. If you have any questions, please contact info@neb.com.

SECTION 3–HAZARDOUS IDENTIFICATION

Emergency Overview: Warning: May cause irritation to skin, eyes, and respiratory tract, may affect kidneys, blood and liver.

HMIS and NFPA Ratings: 0 – Minimal or None, 1 – Slight, 2 – Moderate, 3 – Serious, and 4 – Severe

Health: 1
Flammability: 1
Reactivity: 0

SECTION 4–FIRST AID MEASURES

Eyes: Flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids. Call a physician.

Skin: Wash skin with soap and copious amount of water.

Ingestion: If the person is conscious, wash out mouth with water. Call a physician.

Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SECTION 5–FIRE FIGHTING MEASURES

Extinguishing Media: Water spray. Carbon dioxide, dry chemical powder or appropriate foam.

Special Fire Fighting Procedures: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Fire and Explosion Hazards: Combustible liquid. Emits toxic fumes under fire conditions.

SECTION 6–ACCIDENTAL RELEASE MEASURES

Personal Precautions: Avoid breathing or contact with vapors, mist or gas.

Procedure of Personal Precaution: Wear self-contained breathing apparatus, rubber boots and heavy rubber gloves and chemical safety goggles. Use non-sparking tools and equipment. Ventilate and evacuate area of leak or spill.

Environmental Precautions: Do not let product enter drains.

Methods For Cleaning Up: Cover with dry lime, sand, or soda ash. Sweep up and shovel. Place in covered container for disposal.

SECTION 7–HANDLING AND STORAGE

Handling: Provide appropriate exhaust ventilation.

User Exposure: Avoid inhalation. Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

Storage: Keep tightly closed in a dry and well ventilated place. Store at -20°C .

SECTION 8–EXPOSURE CONTROLS/PPE

Engineering Controls: Safety shower and eye wash. Mechanical exhaust.

Personal Protective Equipment

Eye Protection: Safety goggles.

Hand Protection: Compatible resistant gloves.

Respiratory Protection: Government approved respirator.

Hygiene Measure: General practice, wash (hands and skin) thoroughly after handling. Remove and wash contaminated clothing.

SECTION 9–PHYSICAL AND CHEMICAL PROPERTIES

Physical State: Form: Liquid Color: Clear or colorless Odor: No Data Available

Property	Value	Temperature or Pressure	
Boiling Point Range:		>189°C	
Melting Point Range:		>18.4°C	
Flash Point:		>87°C	Method: Closed cup
Auto Ignition Temp:		>215°C	
Vapor Pressure:	.42 mmHg	20°C	
Vapor Density:	2.7 g/l		
Specific Gravity:	1.1		
Solubility in Water:	Soluble		

SECTION 10–STABILITY AND REACTIVITY

Stability: Stable under recommended storage conditions.

Conditions to Avoid: Moisture

Materials to Avoid: Acid chlorides, Phosphorus halides, strong oxidizing agents, strong acids, strong reducing agents.

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Sulfur dioxides.

Hazardous Polymerization: Will not occur.

Hazardous Exothermic Reactions: Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermicreaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phorphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver flioride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan and sulfur dioxide.

SECTION 11–TOXICOLOGICAL INFORMATION

Acute and Chronic Affects Based On Routes Of Exposure

Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).
Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Specific Developmental Abnormalities: Musculoskeletal System

Eye Contact: May cause irritation.

Skin Contact: May cause irritation.

Ingestion: May cause nausea, coughing, headache or diarrhea.

Inhalation: Unlikely at room temperature, inhalation of mist may cause irritation of respiratory tract.

Chronic Exposure

Target Organ(s): May cause kidney and liver damage.

Aggravation of Pre-existing Conditions: Persons with pre-existing skin disorder or eye problems or impaired liver or kidneys may be more susceptible to the effects of the material.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen.

IARC: No component of this product present at levels greater than or equal to 0.1 % is identified as probable, possible or confirmed human carcinogen.

ACGIH: No component of this product present at levels greater than or equal to 0.1 % is identified as a known or suspected human carcinogen or confirmed animal with unknown relevance humans.

Route of Exposure
Skin Absorption: May be harmful if absorbed.
Contact: May cause skin irritation.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.
Conditions Aggravated By Exposure: Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

Target Organ (s) or System (s): Eyes and Skin

Toxicity Data

Inhalation
Rat
40,250 ppm
LC50

Oral
Rat
3,300 mg/kg
LD50

Oral
Rat
14,500 mg/kg
LD50

Remarks: Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Hemorrhage. Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Conjunctive irritation.

Skin
Rat
40,000 mg/kg
LD50

Intraperitoneal
Rat
8,200 mg/kg
LD50

Subcutaneous
Rat
12 gg/kg
LD50

Remarks: Behavorial: Change in motor activity (specific assay), Lungs, Thorax, or Respiration: Dyspnea.

Intravenous
Rat
5,360 mg /kg
LD50

Remarks: Behavorial: Tremor, Muscle weakness. Lungs, Thorax or Respiration: Dyspnea.

Chronic Exposure - Carcinogen
Species: Rat
Route of Application: Oral
Dose: 59 gm/kg
Exposure Time: 81W
Frequency: I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Species: Rat
Route of Application: Subcutaneous
Dose: 220 gm/kg
Exposure Time: 82W
Frequency I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Chronic Exposure - Mutagen
Species: Rat
Route: Intraperitoneal
Dose: 25 gm/kg
Exposure Time: 5D
Mutation Test: Cytogenetic analysis.

Chronic Exposure - Reproductive Hazard
Species: Rat
Dose: 56 gm/kg
Route of Application: Intraperitoneal
Exposure Time: (6–12D PREG)
Result: Effects on Fertility: Abortion

Species: Rat
Dose: 6,600 mg/kg
Route of Application: Intraperitoneal
Exposure Time: (7–15D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species : Rat
Dose: 30,750 mg/kg
Route of Application: Subcutaneous
Exposure Time: (8–10D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Litter size (e.g.; # fetuses per litter; measured before birth).

SECTION 12–ECOLOGICAL INFORMATION

Elimination Information (persistence and degradability): No data available.

Ecotoxicity Effects

Toxicity to fish	LC50-Pimephales promelas (fathead minnow) - 34,000 mg/l - 96 h
	LC50-Oncorhynycus mykiss (rainbow trout) - 35,000 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	EC50-Daphnia pulex (water flea) - 27,500 mg/l
Toxicity to algae	EC50 - Lepomis macrochirus (Blue Gill) - > 400,000 mg/l - 96 h

Further Information On Ecology: No data available.

SECTION 13–DISPOSAL CONSIDERATIONS

Dispose of container, unused contents and contaminated packaging in accordance with federal, state and local requirement. Contract with a licensed Chemical Waste Disposal Service.

SECTION 14–TRANSPORT INFORMATION

This product is not dangerous and no special precautions are needed according to DOT, ADR/RID (cross border), IMDG and IATA/ICAO.

SECTION 15–REGULATORY INFORMATION

OSHA Hazards: None known.

US Classification and Label Test

US Statements: Combustible. Readily absorbed through skin. Target Organ (s): Eyes, skin, liver and kidneys. Caution. Avoid contact and inhalation.

United States Regulatory Information:
Sara Listed: No

TSCA Inventory Item: Yes

Canada Regulatory Information
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all the information required by the CPR.

DSL: Yes

NDSL: No

EU Additional Classification

S: 23 24/25
Safety Statements: Do not breath vapor. Avoid contact with skin and eyes.

SECTION 16–OTHER INFORMATION

DISCLAIMER

The information provided on the MSDS is furnished in good faith and based on our present knowledge. However, this MSDS shall not constitute a guarantee of any kind. Personnel handling this material must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of this material and the safety and health of employees and customers. NEB assumes no additional liability or responsibility resulting from the use of, or reliance on this information. This product is for R&D use only. Not for drug, household or other uses.

Questions about the information found on this MSDS should be directed to info@neb.com.

Allan Lab Human Cell lines

Human Breast Cancer lines:

21NT – Not commercial available (No MSDS)
21PT - Not commercial available (No MSDS)
MC7
MDA-MB-231
MDA-MB-435S
MDA-MB-468
SUM 149 PT
SUM 159PT
SUM 1315MO2
T47D
MCF 10a
SKBR3

Human Prostate Cancer lines:

VCap
LNCap

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 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.
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: 8166088]. 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%

Related Links

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Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	Price:	$256.00
Designations:	MDA-MB-231	Depositors:	R Cailleau
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	

Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
	Related Cell Culture Products
Applications:	transfection host (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.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 1-2 PGM1, 1-2 PGM3, 1

Cell Biology

ATCC® Number:	**HTB-129™** Order this Item	Price:	**$264.00**
Designations:	MDA-MB-435S		
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
			spindle shaped
Organism:	*Homo sapiens* (human)	Morphology:	PHOTO
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion		
Cellular Products:	tubulin; actin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Isolation:	**Isolation date:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 2

Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	Price:	**$264.00**
Designations:	MDA-MB-468	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		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
	Related Cell Culture Products		
Isolation:	**Isolation date:** 1977		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Receptors:	epidermal growth factor (EGF) transforming growth factor alpha (TGF alpha)		
Tumorigenic:	Yes		
Antigen Expression:	Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)		
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 12 D16S539: 9 D5S818: 12 D7S820: 8 THO1: 7 TPOX: 8,9 vWA: 18		
Cytogenetic Analysis:	modal number = 64; range = 60 to 67. The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.; A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes.; Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, A GLO-I, 1-2 Me-2, 1-2		

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SUM149
P78
3/12/2007
Vial 8
C88
Breast Cancer Line

Sample Search [Go]

Home > Cell Lines > Human Breast Cancer >

SUM-149PT

- Cell Lines
- Human Serum
- Primary Cells
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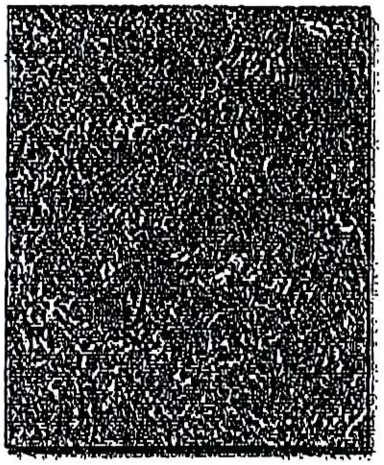


Price
$7,000.00
Academic Price:
$500.00
Your price:
$500.00

Qty: 1

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Invasive Ductal Carcinoma, Inflammatory
ER/PR Status	ER-/PR-
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin and Hydrocortisone added.
Oncogene amplification	None
TGF-beta response	Very sensitive
ERBB receptor status	EGFR ++ (activated) ERBB-2,3 +ve (not activated) ERBB-4 -ve

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer Cell Lines

Cell Line: SUM149PT

Product Description: The SUM149 cell line was developed from Invasive Ductal Carcinoma from a patient with ER negative and PR negative, inflammatory breast cancer. The cell line is immortal and expresses luminal cytokeratins 8, 18, and 19 consistent with their origin from luminal breast epithelial cells. SUM149 has been known to form tumors in nude mice.

Quality control: The cells are grown in antibiotics free medium and monitored for bacterial contamination. The cell cultures are also tested for mycoplasma contamination. One test vial from each lot is thawed and recultured to test for contamination and growth.

Contents and Storage: One vial of 1×10^6 cells in freezing media.

Handling: Upon receiving the frozen cells from Asterand, it is essential that the user consult the enclosed data CD for technical advice regarding cell culture and obtain the necessary growth media and supplements before propagating the cells.

Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them. We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability.

The cells are shipped frozen on dry ice. If you notice any damage to the package or the cells are not frozen, please contact us immediately and if possible send us images of the damage or thawed cells. In this case we will replace the cells free of charge.

If any help is needed to grow the cells please call Asterand's customer service at 313-263-0960 and our scientists will help you over the phone to insure the successful growth of your cells.

Required Cell Culture Media:

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml Medium
Ham's F-12	-	-	500ml bottle
Insulin	1mg/ml	5ug/ml	2.5ml
Hydrocortisone	1mg/ml	1ug/ml	500ul
HEPES	1M	10mM	5ml
Fetal Bovine Serum	-	-	25mls

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 90% confluent. (See Figure 2 below) ~
3. Passage the SUM149 cells at a 1:3 split ratio for the first passage and 1:6 thereafter.

Freezing the cells:

1. Harvest the cells at about 90% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

Additional Information:

SUM149 cells should not be grown to 100% confluency, as they will start to die off and likely not recover.

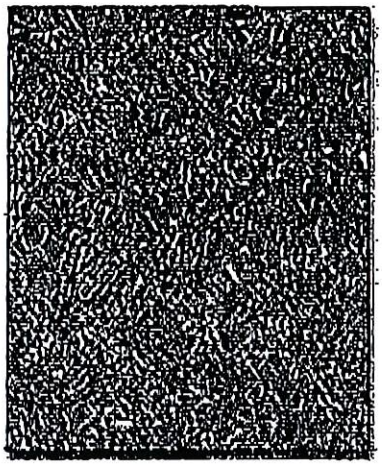
breast cancer sell line derived at the univeristy fo Michigan, known as SUM-159PT

SUM159
P38
3/5/2007
Vial 10
CS5
Breast Cancer line

Sample Search
Go

SUM-159PT

Cell Lines
Human Serum
Primary Cells
RNA
How to Order
FAQ



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Price
$7,000.00
Academic Price:
$500.00
Your price:
$500.00

Qty: 1

Add to Cart

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Anaplastic Carcinoma
ER/PR Status	ER-/PR-
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin & Hydrocortisone added
Oncogene amplification	C-MYC
TGF-beta response	Very sensitive
ERBB receptor status	EGFR +ve, ERBB-2 +ve

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer
Cell Lines

Cell Line: SUM159PT

Product Description: The SUM159 cell line was developed from a primary tumor of a patient with ER negative and PR negative anaplastic carcinoma of the breast. The cell line is immortal and expresses luminal cytokeratins 8, 18, and 19 consistent with their origin from luminal breast epithelial cells. SUM159 has been known to form tumors in nude mice.

Quality control: The cells are grown in antibiotics free medium and monitored for bacterial contamination. The cell cultures are also tested for mycoplasma contamination. One test vial from each lot is thawed and recultured to test for contamination and growth.

Contents and Storage: One vial of 1×10^6 cells in freezing media.

Handling: Upon receiving the frozen cells from Asterand, it is essential that the user consult the enclosed data CD for technical advice regarding cell culture and obtain the necessary growth media and supplements before propagating the cells.

Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them. We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability.

The cells are shipped frozen on dry ice. If you notice any damage to the package or the cells are not frozen, please contact us immediately and if possible send us images of the damage or thawed cells. In this case we will replace the cells free of charge.

If any help is needed to grow the cells please call Asterand's customer service at 313-263-0960 and our scientists will help you over the phone to insure the successful growth of your cells.

Required Cell Culture Media:

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml Medium
Ham's F-12	-	-	500ml bottle
Insulin	1mg/ml	5ug/ml	2.5ml
Hydrocortisone	1mg/ml	1ug/ml	500ul
HEPES	1M	10mM	5ml
Fetal Bovine Serum	-	-	25mls

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

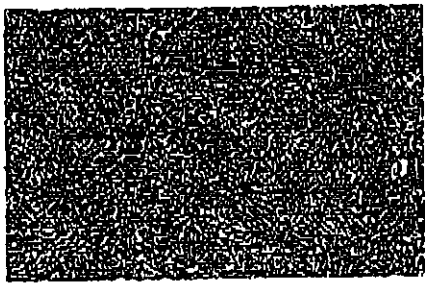
1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 95% confluent.
3. Passage the SUM159 cells at a 1:3 split ratio for the first passage and 1:10 thereafter.

Freezing the cells:

1. Harvest the cells at about 95% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

Additional Information:

The SUM159 lines recover easily from freeze/thaw process. They resemble fibroblasts, and tend to reach confluency in 3-5 days.



SUM159 cells in culture

SUM1316 P37 6/1/2006 Vial 6 Chrystor Breast Cancer

1st vial

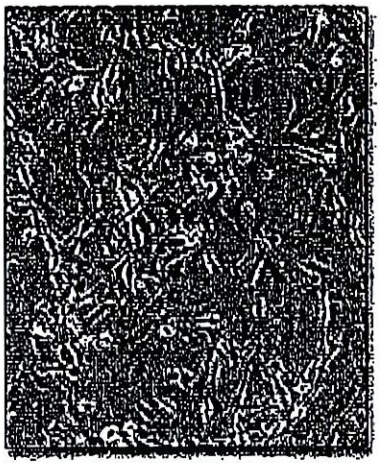
SUM1315 6/13/2006 39 Vial 15

2nd vial received 19 Aug 08

Sample Search Go

SUM-1315MO2

Cell Lines
Human Serum
Primary Cells
RNA
How to Order
FAQ



Price $7,000.00
Academic Price: $500.00
Your price: $500.00

Qty: 1 Add to Cart

All of the cell lines available from Asterand were developed in conjunction with different academic institutions. As a result of our agreements with these institutions, we offer academic and governmental researchers discounted prices.

Description **Additional Information**

Cell Source	Skin Metastasis ductal carcinoma
ER/PR Status	ER-/PR-
Culturo Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin & Epidermal Growth Factor added
Oncogene amplification	None
TGF-beta response	Moderately sensitive
ERBB receptor status	EGFR +ve. Others not determined

Browse for more products in the same category as this item:

Cell Lines > Human Breast Cancer
Cell Lines

Cell Line: SUM1315MO2

Product Description: The SUM1315 cell line was developed from a xenografted metastatic nodule of a patient with invasive infiltrating ductal carcinoma. The cells are immortal and are negative for estrogen and progesterone receptors and express high levels of Her2 and EGF receptor. They have also been shown to form lung and bone metastases after injection into nude mice.

Quality control: The cells are grown in antibiotics free medium and monitored for bacterial contamination. The cell cultures are also tested for mycoplasma contamination. One test vial from each lot is thawed and recultured to test for contamination.

Contents and Storage: One vial of 1×10^6 cells in freezing media.

Handling: Upon receiving the frozen cells from Asterand, it is essential that the user consult the enclosed data CD for technical advice regarding cell culture before propagating the cells.

Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them. We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability.

The cells are shipped frozen on dry ice. If you notice any damage to the package or the cells are not frozen, please contact us immediately and if possible send us images of the damage or thawed cells. In this case we will replace the cells free of charge.

If any help is needed to grow the cells please call Asterand's customer service at 313-263-0960 and our scientists will help you over the phone to insure the successful growth of your cells.

Required Cell Culture Media:

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml Medium
Ham's F-12	-	-	500ml bottle
Insulin	1mg/ml	5ug/ml	2.5ml
EGF	10ug/ml	10ng/ml	500ul
HEPES	1M	10mM	5ml
Fetal Bovine Serum	-	-	25mls

Maintaining the cells:

Reviving the Frozen Cells:

1. Bring the temperature of the culture medium to 37°C
2. Thaw the cells fast (until a small crystal of ice is left) using 37°C water bath, and transfer the cells to a sterile T-25 culture flask containing the above growth medium, and incubate at 37°C humidified tissue culture incubator with 5% CO2 gas supply.
3. Feed the cells three times per week.

Maintain the Cell Culture:

1. Completely change the culture medium the day after initiation and every Monday, Wednesday and Friday thereafter.
2. Subculture the cells when they are 90% confluent. (See Figure 2 below)
3. Passage the SUM1315 cells at a 1:3 split ratio.

Freezing the cells:

1. Harvest the cells at about 90% confluent
2. Determine cell number and viability by using Trypan blue staining.
3. Centrifuge the cells at 1500 rpm for 5 mins at room temperature and resuspend in 1ml of freezing medium (recommend CryoStore CS 5 (Biolife Solutions) per 1x10^6 cells).
4. Using a Mr. Frosty or similar freezing device, the cell vials are placed into a 80°C freezer overnight and then stored long term in vapor phase liquid nitrogen.

Additional Information: The SUM1315 cells MUST BE grown in 5% FBS media with insulin and epidermal growth factor as described above. The cells may take two or more passages to recover from the thawing process. Because of the size of the cell, about 1.5x10^6 cells can be expected per 1 T-75 flask.

-------- Original Message --------
Subject: Re: Containment Level - SUM cell lines
Date: Wed, 21 Jan 2009 15:30:07 -0500
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Ms. Stanley,

These cell lines do not seem to have been transformed with any viruses nor contaminated with risk group 2-4 pathogens. They would most probably be risk group 1. To give you a definite answer I would have to do a risk assessment. When you are ready to import, send your application with all the information you can find on these cell lines (origin, how it was immortalized, is it pathogen free, research articles...).

Regards

Genevieve Lacroix
A/Head, Importation and Biosafety Program/
Chef Intérimaire, Importation et Services de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada/ Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 946-6982
Fax: (613)941-0596
genevieve_lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

Jennifer Stanley <jstanle2@uwo.ca>
2009-01-21 02:10 PM

To
genevieve_lacroix@phac-aspc.gc.ca
cc

Subject
Containment Level - SUM cell lines

Hello Genevieve:

Can you advise me on the containment level for these cells (ie if we decide to import them)?

Thanks
Jennifer

SUM 149PT:
http://www.asterand.com/Asterand/human_tissues/149PT.htm

SUM159:
http://www.asterand.com/Asterand/human_tissues/159PT.htm

SUM1315MO2:
http://www.asterand.com/Asterand/human_tissues/1315M02.htm

Designations: T-47D
Depositors: I Keydar
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Homo sapiens* (human)
Morphology: epithelial

Source:
Organ: mammary gland; breast
Tissue: duct
Disease: ductal carcinoma
Derived from metastatic site: pleural effusion

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 Transfection Reagents)

Receptors:
calcitonin, expressed
androgen receptor, expressed
estrogen receptor, expressed
progesterone receptor, expressed
glucocorticoid receptor, positive, expressed
prolactin, expressed
calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin; estrogen receptor, positive

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11,13
D13S317: 12
D16S539: 10
D5S818: 12
D7S820: 11
THO1: 6
TPOX: 11
vWA: 14

Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number is 65 occurring at 50% and polyploidy at 0.8%. 18 marker chromosomes are common to most cells, of which 7 are paired and 11 are single-copied. The t(8q14q), t(9q17q), t(10q17p) are among 7 paired markers common to most cells. N7, N9, and N10 are absent and N11 is generally present in 4 copies. DM's occurred, but infrequently. Q-band examination did not show the presence of a Y chromosome.

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

Age: 54 years adult

Gender: female

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].
The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast.
This differentiated epithelial substrain (T-47D) was found to contain cytoplasmic junctions and receptors to 17 beta estradiol, other steroids and calcitonin.

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: 0.2 Units/ml bovine insulin; fetal bovine serum to a final concentration of 10%

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Product Information Sheet

Designations:	MCF 10A
Depositors:	Michigan Cancer Foundation
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	Organ: mammary gland; breast Disease: fibrocystic disease 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: August 22, 1984
Applications:	transfection host (Roche Transfection Reagents)
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 8,9 D16S539: 11,12 D5S818: 10,13 D7S820: 10,11 THO1: 8,9.3 TPOX: 9,11 vWA: 15,17
Isoenzymes:	AK-1, 1 [23084] ES-D, 1 [23084] G6PD, B [23084] GLO-I, 1-2 [23084] PGM1, 1-2 [23084] PGM3, 1 [23084]
Age:	38 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF 10A cell line is a non-tumorigenic epithelial cell line. [21968] The line was produced by long term culture in serum free medium with low Ca++ concentration. [21968] MCF 10A was derived from adherent cells in the population. [21968] Cells derived from a floating population are available (see MCF 10F, ATCC CRL-10318). [21968] The cells are positive for epithelial sialomucins, cytokeratins and milk fat globule antigen. [21968] They exhibit three dimensional growth in collagen, and form domes in confluent cultures. [21968] Thus far, the cells have shown no signs of terminal differentiation or senescence. The line is responsive to insulin, glucocorticoids, cholera enterotoxin, and epidermal growth factor (EGF). [21968] By electron microscopy the cells display characteristics of luminal ductal cells but not of myoepithelial cells. [23085] They also express breast specific antigens as detected by positive reaction with MFA-Breast and MC-5 monoclonal antibodies. [23085] The calcium content of the medium exerts a strong effect on the morphology of the cells. [22248]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line (MEGM) along with the additives can be obtained from Lonza/Clonetics Corporation as a kit: MEGM, Kit Catalog No. CC-3150. ATCC does not use the GA-1000 (gentamycin-amphotericin B mix) provided with kit. To make the complete growth medium, you will need to add the following components to the kit (sold separately): - 100 ng/ml cholera toxin Note: Do not filter complete medium Temperature: 37.0°C

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Product Information Sheet

Designations:	SK-BR-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: mammary gland; breast Disease: adenocarcinoma Derived from metastatic site: pleural effusion
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: 1970
Applications:	transfection host (Nucleofection technology from Lonza Roche Transfection Reagents)
Tumorigenic:	Yes
Antigen Expression:	Blood Type A; Rh+; HLA A11, Bw22(+/-), B40, B18
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 11,12 D16S539: 9 D5S818: 9,12 D7S820: 9,12 THO1: 8,9 TPOX: 8,11 vWA: 17
Cytogenetic Analysis:	This is a hypertriploid human cell line with the modal chromosome number of 84, occurring in 34% of cells. Cells having 80 chromosomes also occurred at a high rate (28%); the higher ploidy cells occurred at 7.3%. This cell line has a very complex chromosome composition. Thirty-five to 40% of chromosomes in a cell complement with a modal chromosome number of 84 consisted of structurally altered marker chromosomes. Several markers are longer than chromosome N1. The origins of most of these markers, however, are not clear. Some markers may have at least three individual chromosome segments. The markers [i.e., 7der(1)t(1;21) (p13;q21) [or 7t(1q21q)], 7del(2) (q13), and t(7ptor—cen—?), present in some cells only] were the only ones in which portions of chromosome segments could be identified. Most cells had about three normal X chromosomes and five or more N7. The structurally normal N1, N14 and N17 were generally absent.
Isoenzymes:	AK-1, 1-2 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 1-2 PGM3, 1
Age:	43 years
Gender:	female
Ethnicity:	Caucasian

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Product Information Sheet

ATCC: Catalog Search

Designations:	VCaP
Depositors:	KJ Pienta
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	Organ: prostate Tissue: vertebral metastasis Disease: cancer 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: 1997
Tumorigenic:	Yes
Antigen Expression:	cytokeratin-18; Homo sapiens, expressed p53 antigen; Homo sapiens, expressed prostate specific antigen (PSA); Homo sapiens, expressed prostatic acid phosphatase (PAP); Homo sapiens, expressed Rb protein; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11,12 D16S539: 9 D5S818: 12 D7S820: 9,12 THO1: 9.3 TPOX: 8,11 vWA: 18,19
Age:	59 years
Gender:	male
Ethnicity:	Caucasian
Comments:	This line was established in 1997 from a vertebral bone metastasis from a patient with hormone refractory prostate cancer. It was passaged as xenografts in mice then cultured in vitro. It is androgen sensitive in vitro and in vivo.
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:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Hank's Balanced Salt Solution or 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 1.0 to 2.0 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

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Product Information Sheet

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* (human)
Morphology:	epithelial
Source:	**Organ:** prostate **Disease:** carcinoma **Derived from metastatic site:** left supraclavicular lymph node
Cellular Products:	human prostatic acid phosphatase; prostate specific antigen [21889]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	Distribution of this material for commercial purposes will require execution of a Non-exclusive License Agreement. At the time of placing an order, customers must send a request to licensing@ATCC.org. Orders will be shipped when Customer Service receives confirmation from our Licensing officer.
Isolation:	**Isolation date:** 1977
Applications:	transfection host (Nucleofection technology from Lonza Roche Transfection Reagents)
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
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. [21889] These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production). [23045] 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

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Product Information Sheet

Cell Biology

ATCC® Number:	**CRL-2539™**	Order this Item	Price:	**$279.00**

Designations:	4T1
Depositors:	BA Pulaski
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	epithelial
Source:	**Organ:** mammary gland **Strain:** BALB/cfC3H **Disease:** tumor
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.
Tumorigenic:	Yes
Comments:	4T1 is a 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment. [49690] When injected into BALB/c mice, 4T1 spontaneously produces highly metastatic tumors that can metastasize to the lung, liver, lymph nodes and brain while the primary tumor is growing in situ. [49688] [49690] The primary tumor does not have to be removed to induce metastatic growth. The tumor growth and metastatic spread of 4T1 cells in BALB/c mice very closely mimic human breast cancer. This tumor is an animal model for stage IV human breast cancer. [49688] [49689] 4T1-induced tumors can be used as a post-operative model as well as a non-surgical model because the 4T1-induced tumor metastasizes spontaneously in both models with similar kinetics. [49687] [49688] [49689] Because 4T1 is resistant to 6-thioquanine, micro-metastatic cells (as few as 1) can be detected in many distant site organs with better accuracy that most tumor models. There is no need to count nodules or weight target organs. [49687] [49688] [49689]
Propagation:	

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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

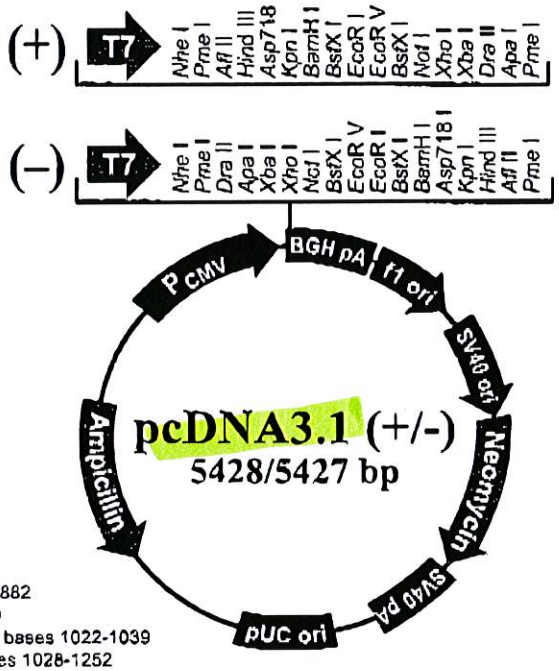
BioStandards

Biological Reference Material and Consensus Standards for the life science community

Appendix
pcDNA™3.1 Vectors

Map

The figure below summarizes the features of the pcDNA™3.1(+) and pcDNA™3.1(–) vectors. **The complete sequences for pcDNA™3.1(+) and pcDNA™3.1(–) are available for down-loading from our World Wide Web site (www.invitrogen.com) or from Technical Support (see page 13).** Details of the multiple cloning sites are shown on page 3 for pcDNA™3.1(+) and page 4 for pcDNA™3.1(–).



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

continued on next page

invitrogen™ **Material Safety Data Sheet** **Revision Date:** 29-Apr-2010

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

Product code 350484
Product name pcDNA3.1/(+)

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

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

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

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

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

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

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

For research use only

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

End of Safety Data Sheet

Info on Toxin(s)

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 4.2
Revision Date 11/05/2010
Print Date 08/17/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Cholera Toxin Vibrio cholerae

Product Number : C8052
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Bowel

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects — Moderate skin irritant; Moderate eye irritant

GHS Classification

Acute toxicity, Oral (Category 5)
Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity - single exposure (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H303 May be harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

Precautionary statement(s)

P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HMIS Classification

Health hazard: 2

Chronic Health Hazard: *
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	Harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.
Ingestion	Harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cholera enterotoxin
Cholergen

CAS-No.	EC-No.	Index-No.	Concentration
Tris (hydroxymethyl) aminomethane			
77-86-1	201-064-4	-	>= 5.82 - <= 5.94 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	>= 31.3 - <= 31.9 %
Sodium chloride			
7647-14-5	231-598-3	-	>= 57.6 - <= 58.8 %
Exotoxin, vibrio cholerae			
9012-63-9	-	-	>= 0.5 - <= 2.5 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	>= 0.96 - <= 0.98 %

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting/freezing	no data available

point	
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Dimethyl sulfate, Acid chlorides, Halogenated hydrocarbon, Metals, Acids

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Ingestion	Harmful if swallowed.
Skin	Harmful if absorbed through skin. Causes skin irritation.
Eyes	Causes eye irritation.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Exotoxin, vibrio cholerae	9012-63-9

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate eye irritant

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera
Proposed Use Dose:	50 µg
Proposed Storage Dose:	500 µg
LD_{50} (species):	250 µg

Calculation:

250 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 12500

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 1250

Comments/Recommendations:

Assume density of cholera is 1 g/mL.

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

Canada

Containment Level 2 Physical and Operational Requirements in Accordance with the Laboratory Biosafety Guidelines, 3rd Edition, 2004

Facility Information

Office use only
File # :

Human Pathogens and Toxins Act (HPTA) Registration Number*:

R-06-000599

Facility Name:

Cancer Research Laboratory Program, London Health Sciences Centre

Room number(s) or name(s) where pathogen(s) will be manipulated and/or stored:

A4-114, A4-116, A4-128, A4-822, A4-824, A4-826, A4-903, A4-908, A4-910

Laboratory Physical Address (Not a Post Office Box):

London Regional Cancer Program
London Health Sciences Centre
790 Commissioners Road East

City:

London

Province:

Ontario

Postal Code:

N6A 4L6

Mailing Address: Same as Laboratory Physical Address ☒

City:

Province:

Postal Code:

Type of Facility

☐Government (Federal) ☒University ☐Private ☐Government (Provincial) ☒ Hospital ☐ Other

Program Intent – Brief description of the type of work and program objectives (research, diagnostic, production).

Basic research involving the following: use of cloning vectors and mammalian cell lines; ranslational cancer research using human tumour specimens; growth of human adenovirus in human tumour cell lines; transfection of human tumour cell lines using lentivirus system; growth of lentivirus-transfected cell lines.

Scale/Volume

☒Laboratory ☐Large Scale ☐ Other _____

Comments

The certified rooms are tissue culture rooms.

Pathogens used and/or stored

Affecting humans : ☒ Yes ☐ No Affecting animals/fish: ☐ Yes ☒ No

Comments

*Your Human Pathogens and Toxins Act (HPTA) Registration Number is available on your HPTA Registration Letter. Consult your Biosafety Officer for further information.

Containment Level 2 Checklist

Adenoviruses are ubiquitously found agents that can infect humans of all ages. The infection is generally of limited symptoms, but can be dangerous in immunocompromised patients. Lentivirus is used to transfect expression vectors into cell lines. The virus does incorporate into the genome of infected cells, requiring caution with its use. However, the transformed cells do not produce or shed virus. The use of lentivirus is confined to room A4-822, and use is limited to personnel trained in the use of this virus.

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

Canada

Containment Level 2 Checklist

List of pathogens: (species and subtypes where applicable)

mycoplasma pneumoniae; lentivirus; adenovirus

Use of Animals: ☐ Yes ☒ No

Species and Quantity:	

Contact Information

Facility Supervisor (main contact†)

Name: Dr. James Koropatnick

Title: Professor

Department: Physiol. & Pharmacol., Micro. & Immuno., Oncol.

Address: Room A4-107, LRCP-LHSC, 790 Commissioners Road E., London, N6A 4L6

Telephone: 519-685-8600 Ext 58654

Fax: 519-685-8616

Email: jkoropat@uwo.ca

Language Preference: ☒ English ☐ French

Other Comments:

Signature: James Koropatnick Date: Dec. 10, 2010

Biosafety Officer (or equivalent)

Same as Facility Supervisor ☐

Name: Gail Ryder

Title: Biosafety Officer

Department: Lawson Health Research Institute - LHSC

Address: Room A210, NR, 375 South St., LHSC-SS, London, N6A 4G5

Telephone: 519-685-8500 Ext 75109

Fax: 519-432-7367

Email: Gail.Ryder@LawsonResearch.com

Language Preference: ☒ English ☐ French

Other Comments:

Signature: Gail Ryder Date: Dec 10, 2010

Compliance letters should be sent‡ to (select only one option): ☒ Main Contact; ☒ Biosafety Officer

† Note: compliance letters will be issued in the name of the Main Contact entered on this page.

‡ Note: compliance letters can be sent to either the Main Contact or the Biosafety Officer. If left blank or if both options are selected, the documents will be sent, by default, to the Main Contact.

------- Original Message --------
Subject: Re: Biological Agents Registry Form (Allan)
Date: Fri, 26 Aug 2011 15:19:43 -0400
From: Alison Allan <Alison.Allan@lhsc.on.ca>
To:jstanle2@uwo.ca

Hi Jennifer--

Thanks for your email. I have indicated answers to your questions below. Let me know if you need any further information.

regards,
Alison

>>> Jennifer Stanley 08/26/11 3:07 PM >>>
Hi Dr. Allan

I received your form today. I have a couple of questions:

Table 4.2 - Do you have any information, such as an MSDS, on the NEB 5a competent E. coli? Where do you get it from?
Please see attached for the MSDS sheet. The E.coli were obtained from New England Biolabs (NEB)

Question 6.4 - what cell line(s) do you inject into mice (it says "both listed")
The two cell lines that were injected were the two transfected ones listed in the table (MDA-MB-468/pcDNA3.1 and MDA-MB-468/OPN)

I noticed that you had a PHAC checklist attached, dated December 2010. It was in Dr. Koropatnick's name so I guess it was included because you share a lab with him???
1. Was the compliance letter received?
2. I gather than the Koropatnick lab (not your lab) uses adenovirus and lentivirus?
3. Normally PHAC checklists are done to get an import permit . I gather that the Koropatnick lab (not your lab) was importing items - as Section 11.0 was NO on your form.
I work in the open concept lab space on the 4th floor of the Victoria Research Labs, along with 7 other scientists.
Dr. Koropatnick (as Director of the Cancer Research Laboratories) is responsible for the space, that is why the permit is in his name.
We do not work with adenovirus or lentivirus, although several other scientists in the space do.
You will have to check with Gail Ryder with regards to the compliance letter.

Have a great weekend.

Regards,
Jennifer