

**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 | <u>James R Hammond</u> |
| DEPARTMENT | <u>Physiology and Pharmacology</u> |
| ADDRESS | <u>M266 Medical Sciences Bldg</u> |
| PHONE NUMBER | <u>83780</u> |
| EMERGENCY PHONE NUMBER(S) | <u>519-661-0003</u> |
| EMAIL | <u>jhammo@uwo.ca</u> |

Location of experimental work to be carried out: Building(s) _MSB_ Room(s) _271/295_

*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: _NSERC, CIHR, HSFO_

GRANT TITLE(S): _HSFO: Role of nucleoside/nucleobase transporters in the regulation of the vascular effects of adenosine and its metabolites. NSERC: Regulation of nucleoside transporters. CIHR: Structure function analysis of drug interactions with nucleoside transporters

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

| <u>Name</u> | <u>UWO E-mail Address</u> | <u>Date of Biosafety Training</u> |
|---------------------------|----------------------------|-----------------------------------|
| <u>Derek Bone</u> | <u>derekbone@gmail.com</u> | <u>Sept 2004</u> |
| <u>Jamie Park</u> | <u>jpark44@uwo.ca</u> | <u>Sept 2007</u> |
| <u>Frances Cunningham</u> | <u>fcunnin2@uwo.ca</u> | <u>Sept 2008</u> |
| <u>Scott Hughes</u> | <u>shughe3@uwo.ca</u> | <u>June 2009</u> |
| <u>Samer Serhan</u> | <u>samy1000@gmail.com</u> | <u>Sept 2010</u> |
| <u>Diana Quinonez</u> | <u>dquinon@uwo.ca</u> | <u>Sept 2009</u> |
| <u>Samantha Li</u> | <u>sli288@uwo.ca</u> | <u>June 2010</u> |
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Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

1. E coli bacteria strains are used for the replication of plasmid DNA for molecular biology work
2. Mammalian cell lines are used for the characterization of nucleoside transport and adenosine metabolism. Cells are grown in typical tissue culture flasks/plates and harvested by trypsinization for use in functional transport assays.
3. PK15-NTD cells are a mutant cell line that is deficient in nucleoside transport, and hence are used as a null-background model for the transfection of mutant nucleoside transporters for subsequent functional characterization.

Cells stocks, when not actively growing in 5% CO₂/humidified cell incubators, are maintained in liquid nitrogen. All materials from studies done with these cells are handled as biohazardous waste and autoclaved and disposed of as specified by OHS.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? YES NO

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

What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

| 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 |
|--|--|--|--|---|------------------|---|
| XL-1 blue | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | 0.1 litre | Stratagene | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| DH5alpha | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | <input type="radio"/> Yes <input checked="" type="radio"/> No | 0.1 litre | Invitrogen | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | <input type="radio"/> Yes <input type="radio"/> No | | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |

*Please attach a Material Safety Data Sheet or equivalent for each agent.

E. coli - Level 1

2.0 Cell Culture

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

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

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|-------------------|---|--|---------------------|
| Human | <input checked="" type="radio"/> Yes <input type="radio"/> No | Heart (purchased from Lonza) | |
| Rodent | <input checked="" type="radio"/> Yes <input type="radio"/> No | Microvascular endothelial cells from mouse skeletal muscle | 2009-030 |
| Non-human primate | <input type="radio"/> Yes <input checked="" type="radio"/> No | | |
| Other (specify) | <input type="radio"/> Yes <input checked="" type="radio"/> 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 | <input checked="" type="radio"/> Yes <input type="radio"/> No | HEK293 U2_OS HMEC-1 | 2 1 1 | ATCC ATCC CDC |
| Rodent | <input checked="" type="radio"/> Yes <input type="radio"/> No | UMR108 C2C12 | 1 1 | ATCC ATCC |
| Non-human primate | <input type="radio"/> Yes <input checked="" type="radio"/> No | | | |
| Other (specify) | <input checked="" type="radio"/> Yes <input type="radio"/> No | PK15-NTD (pig) | 1 | Ming Tse, Johns Hopkins Univ |

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

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

3.0 Use of Human Source Materials

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

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

| Human Source Material | Source/Supplier /Company Name | Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN | Name of Infectious Agent (If applicable) | PHAC or CFIA Containment Level (Select one) |
|--|-------------------------------|--|--|---|
| Human Blood (whole) or other Body Fluid | | <input type="radio"/> Yes <input type="radio"/> Unknown | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| Human Blood (fraction) or other Body Fluid | | <input type="radio"/> Yes <input type="radio"/> Unknown | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3 |
| Human Organs or Tissues (unpreserved) | | <input type="radio"/> Yes <input type="radio"/> Unknown | | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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 1.0 and 2.0? YES NO If no, please

4.2 ⇒ Yes
→ E. coli

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 Transfected | Describe the change that results from transformation or tranfection |
|-----------------------------|---------------------------|--------------------------------|------------------|--|
| XL-1 Blue DH5-alpha | pcDNA3.1 p3*FLAG-CMV10 | Sigma-Aldrich Sigma-Aldrich | hENT1 hENT1 | .Change in nucleoside transport capacity – leads to modification of cell growth rate |

* 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?

YES, complete table below NO

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used C57BL/6 Mice

6.3 AUS protocol # 2009-030

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

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)? YES No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

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

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ 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? YES NO If no, please proceed to Section 9.0

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

8.3 What is the LD₅₀ (specify species) of the toxin _____

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

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ 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? YES NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____
 "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? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? YES 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? YES 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? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

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

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

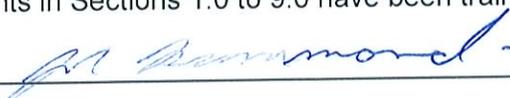
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus: BIO-UWO-0195
 NO, please certify
 NOT REQUIRED for Level 1 containment

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.
N/A

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:
Immediate wash with warm water and soap, then referred to Health Services for followup

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 [Signature] Date: Nov 29, 2010

15.0 Approvals

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

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

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

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

Special Conditions of Approval:

Please include a one page research summary or teaching protocol.

Nucleoside transporters are critical for the cellular uptake and release of endogenous nucleosides. They are also important in the cellular uptake, and thus therapeutic activity, of a range of nucleoside analogues used to treat cancer and viral diseases. Our studies examine the mechanisms by which these membrane proteins transport substrates and bind agents that inhibit nucleoside uptake.

Structure-function analysis of nucleoside transporters (funded by CIHR): To obtain information on the parts of the protein responsible for substrate translocation and inhibitor binding, we are undertaking site directed mutagenesis studies on the ENT1 subtype of nucleoside transporter. Specifically, we are using cysteine scanning mutagenesis approaches to determine the amino acid residues important to the sensitivity of the function of this transporter to sulfhydryl reagents. Mutant ENT1 proteins are expressed in PK15-NTD cells and examined for their affinity for nucleoside substrates and for the sensitivities to inhibition by compounds such as nitrobenzylthioinosine, dipyridamole and draflazine. These studies will lead to the identification of the 'pharmacophore' of the ENT1 transporter, and guide the development of new, more efficacious substrates and inhibitors.

Regulation of nucleoside transporters (funded by NSERC and HSFO): This project involves the examination of the role of protein kinase C and/or protein kinase CK2 in the regulation of the plasma membrane expression of nucleoside and nucleobase transporters. It is well established that protein kinase manipulation can affect the cellular expression of nucleoside transporters, and that this regulation is linked to the cell cycle and the rate of cell growth and proliferation. We are using two approaches to examine this regulation. First of all, we are conducting site-directed mutagenesis to remove each of the potential serine/threonine residues of ENT1 that are predicted to be sites of phosphorylation by protein kinases. These mutants are then expressed in PK15-NTD cells to determine the sensitivity of the mutant transporters to agents which inhibit or activate protein kinases in these cells. The second model involves the use of microvascular endothelial cells isolated from human cardiac tissue (purchased from commercial source) or from mouse skeletal muscle (wild-type and ENT1-null mice; primary cultures obtained in-house). These cells typically line the walls of the capillary networks which are involved in the local regulation of tissue blood flow. It is well established that adenosine, the primary endogenous substrate for these transporters, is involved in the regulation of blood flow as a vasodilator. How the vasculature handles adenosine is an important avenue of investigation which may lead to more evidence-directed therapeutic interventions in cardiovascular diseases. We are examining the role of various protein kinase signaling pathways and growth factor receptor mediated signaling pathways (e.g. VEGF) in the regulation of the nucleoside and nucleobase transporters of these cells.

CERTIFICATE OF ANALYSIS

Product Code: CC-7030
Product: HMVEC-C Cardiac MV Endo
 Cells,EGM-2MV, cryo amp

Lot Number: 7F3303
Manufacture Date: 16-Mar-2007

| TEST (Method) | SPECIFICATIONS | | Results |
|--------------------------------|-----------------|-------|--------------|
| | Min. | Max. | |
| Tissue Acquisition Number | *** | *** | 15429 |
| Donor Screen Information: | | | |
| Age | *** | *** | 12 Y |
| Race | *** | *** | B |
| Sex | *** | *** | MALE |
| Cell Type | *** | *** | HMVEC-C |
| QC Evaluation Medium | | | EGM-2MV |
| Date of Cryopreservation | *** | *** | 16 MAR 2007 |
| Cell Strain Calculations: | | | |
| Cell Passage | | | 3 |
| Viability-Tryp.Blue Exclusion | > =-70% | ***** | 87 % |
| Cell Count (Cells/ml) | > =500,000 | ***** | 622500 |
| Total Population Doublings | > =10 | ***** | 15 |
| Seeding Efficiency | > =20% | ***** | 41 % |
| Doubling Time (hours) | 15 | 48 | 34 |
| Sterility Test | *** | *** | Negative |
| Mycoplasma | *** | *** | Negative |
| Virus Testing: | | | |
| HIV Test | ***** | ***** | Not detected |
| HBV Test | ***** | ***** | Not detected |
| HCV Test | ***** | ***** | Not detected |
| Acetylated LDL Uptake Staining | > =70% Positive | ***** | Pass |
| Alpha Actin Expression | < =10% Positive | ***** | Pass |
| Factor VIII Related Antigen | > =70% Positive | ***** | Pass |

For all lot numbers ending in J. This lot has been isolated from human tissue obtained under "informed consent". This lot has been tested in accordance with Lonza's test procedures and sampling plans. Bovine raw materials such as tissues/organs used to manufacture cell culture media, used to manufacture this lot of cells were sourced from New Zealand, which is recognized as being free of BSE by the OIE. We have verified through our suppliers that in the process from the importation into the U.S. as raw materials until the exportation to Japan as reagents the raw material/ reagents have not come into contact with any tissues/ organs or their extracts derived from cattle and sheep in EU countries and those having BSE disease.

This lot has been reviewed by Quality Assurance in compliance with requirements of Lonza's Quality System.
 This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.



Lonza Walkersville Inc.
 Walkersville Warehouse
 8830 Biggs Ford Road
 Walkersville 21793-0127
 Phone:
 Fax:

1 / 1

0004639339

Packing List

0004639339

| | | | |
|-------------------------|----------------------------------|------------------------|--------------------|
| Shipping Point: | Walkersville 21793-0127 | Shipping Terms: | FCA - Free carrier |
| Delivery Date: | 29-Jul-2010 | | |
| Freight Carrier: | FED EX | | |
| Freight Mode: | LBS - Overnight std | | |
| Route: | ZLAIR1-LBS Overnight Air - 1 Day | | |

Customer No.: 5008419

Ship to:

UNIV OF WESTERN ONTARIO
 PHYSIOLOGY & PHARMACOLOGY
 Attn: Hammond
 Dock #15
 DENTAL SCI BLDG Rm 0037
 LONDON ON N6A 5C1
 CANADA

Sold to:

UNIV OF WESTERN ONTARIO
 PHYSIOLOGY & PHARMACOLOGY
 DENTAL SCI STORES
 LONDON ON N6A 5C1
 CANADA

Bill to:

Company
 UNIV OF WESTERN ONTARIO
 ACCTS PAYABLE SUPPORT SERVIC
 1393 WESTERN RD STE 6100
 LONDON ON N6A 3K7
 CANADA

| Order No. | Order Date | Customer Order No. | Customer Contact |
|-----------|-------------|--------------------|------------------|
| 3557959 | 15-Jul-2010 | 538042 | JAMES HAMMOND - |

| Line | Product Code/ Item Description | Order Qty | Ship Qty | UOM | Lot No./Ser.Nr. | Expiration date |
|------|--|--------------|-------------|-----|-----------------|-----------------|
| 050 | CC-7030 HMVEC-C Cardiac MV Endo Cells,EGM-2MV, cryo amp | 2 | 1 | AMP | 7F3303 | |

Delivery Instructions:

Footer Notes:

In the event of an emergency with the products contained in this package, please contact 800-424-9300 or 703-527-3887 (collect calls)

Effective December 31, 2009: purchase orders for products & services from the Cell Discovery & Molecular Biology catalogs must be submitted to one vendor as follows:

Vendor: Lonza Walkersville, Inc., 8830 Biggs Ford Road, Walkersville, MD 21793

Federal ID: 95-3917176

HMVEC - C

Lonza

Lonza Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Walkersville, MD 21793-0127 USA
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Terms and Conditions

Definitions

These Terms and Conditions apply to Lonza Rockland and Lonza Walkersville, herein referred to as "Seller."

Warranty

Due to the various factors affecting research test results, Seller warrants only and not for any particular purpose of the purchases, that all products sold will perform according to established product specifications. Products are sold with the understanding that the purchaser will determine if the product is suitable for his or her application. We will replace any product, free of charge, that does not meet our established product release specifications. Seller shall not be liable for any damages or injury to persons or property arising from the purchase or use of the product. In no event shall Seller's liability exceed the purchase price paid by the buyer. In addition, we are not liable for the product after the product expiration date or for a product that has been misused or has become unusable due to improper storage or handling by purchaser.

Seller guarantees the performance of Clonetics[®] and Poietics[®] cells up to two years from purchase only if appropriate Clonetics[®] or Poietics[®] media and reagents are used exclusively, and the recommended storage and use protocols are followed. Cell and media performance is not guaranteed if any modifications are made to such cell systems.

Product Use

Clonetics[®] and Poietics[®] cell products are intended for research purposes only and the buyer has no rights to transfer the products, components, or materials made using these products, or, use these products for Commercial Purposes. Commercial Purposes includes 1) use of the products or their components in manufacturing; 2) use of the products or their data components to provide a service, information, or data; 3) use of the products or their components for therapeutic or diagnostic purposes; 4) resale of the products or their components.

Safety Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures.

WARNING: CLONETICS[®] AND POIETICS[®] PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Technical Services.

Material Safety Data Sheet



Stratagene XL1-Blue Supercompetent Cells, Catalog #200236

1. Product and company identification

Product name : **Stratagene XL1-Blue Supercompetent Cells, Catalog #200236**

Part No. : pUC18 Control Plasmid 200231-42
 DNA
 1.42 M 2-Mercaptoethanol 210200-43
 XL1-Blue Competent Cells 200236-41

Manufacturer / Supplier : Agilent Technologies, Inc.
 1834 State Highway 71 West
 Cedar Creek, TX 78612

Emergency telephone number : 1-800-894-1304

Use of the substance/preparation : Chemical Kit

Validation date : 01/09/2009



2. Hazards identification

Physical state : pUC18 Control Plasmid Liquid.
 DNA
 1.42 M 2-Mercaptoethanol Liquid.
 XL1-Blue Competent Cells Liquid.

Odor : pUC18 Control Plasmid Not available.
 DNA
 1.42 M 2-Mercaptoethanol Not available.
 XL1-Blue Competent Cells Not available.

OSHA/HCS status : pUC18 Control Plasmid While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
 DNA
 1.42 M 2-Mercaptoethanol This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
 XL1-Blue Competent Cells This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Emergency overview-Signal Word : WARNING !

Emergency overview-Label Statement : pUC18 Control Plasmid NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
 DNA
 1.42 M 2-Mercaptoethanol HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
 XL1-Blue Competent Cells HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

2. Hazards identification

| | | |
|---|---|---|
| | pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol | No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing. Toxic if swallowed. Irritating to eyes and skin. May cause sensitization by skin contact. Do not breathe vapor or mist. Do not ingest. Do not get on skin or clothing. Avoid contact with eyes. Wash thoroughly after handling. |
| | XL1-Blue Competent Cells | Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling. |
| | pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent Cells | Not available. Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea. |
| Routes of entry | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | Eye contact. Ingestion. Dermal contact. Eye contact. Inhalation. Ingestion. Eye contact. Inhalation. Ingestion. |
| <u>Potential acute health effects</u> | | |
| Eyes | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | No known significant effects or critical hazards. Irritating to eyes. No known significant effects or critical hazards. |
| Skin | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | No known significant effects or critical hazards. Irritating to skin. May cause sensitization by skin contact. No known significant effects or critical hazards. |
| Inhalation | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | No known significant effects or critical hazards. No known significant effects or critical hazards. No known significant effects or critical hazards. |
| Ingestion | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | No known significant effects or critical hazards. Toxic if swallowed. Toxic if swallowed. |
| Medical conditions aggravated by over-exposure | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | Not applicable. Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to the substance can produce target organs damage. |
| Over-exposure signs/symptoms | : pUC18 Control Plasmid DNA 1.42 M 2-Mercaptoethanol XL1-Blue Competent Cells | Not applicable. Not applicable. Not applicable. |

See toxicological information (section 11)

3 . Composition/information on ingredients

| <u>Name</u> | <u>CAS number</u> | <u>%</u> |
|---------------------------------|-------------------|----------|
| 1.42 M 2-Mercaptoethanol | | |
| 2-Mercaptoethanol | 60-24-2 | 10 |
| XL1-Blue Competent Cells | | |
| Glycerol | 56-81-5 | 5 - 10 |
| Manganese dichloride | 7773-01-5 | 5 - 10 |
| Sucrose | 57-50-1 | 5 - 10 |
| Dimethyl sulfoxide | 67-68-5 | 5 - 10 |
| Potassium chloride | 7447-40-7 | 1 - 5 |

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

| | | |
|---------------------|-----------------------------|---|
| Eye contact | : pUC18 Control Plasmid DNA | In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe. |
| | 1.42 M 2-Mercaptoethanol | In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe. |
| | XL1-Blue Competent Cells | In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe. |
| Skin contact | : pUC18 Control Plasmid DNA | In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe. |
| | 1.42 M 2-Mercaptoethanol | In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe. |
| | XL1-Blue Competent Cells | In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe. |
| Inhalation | : pUC18 Control Plasmid DNA | If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe. |
| | 1.42 M 2-Mercaptoethanol | If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe. |
| | XL1-Blue Competent Cells | If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe. |

4. First aid measures

| | | |
|-----------------------------------|--|---|
| Ingestion | : pUC18 Control Plasmid DNA | Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe. |
| | 1.42 M 2-Mercaptoethanol | Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe. |
| | XL1-Blue Competent Cells | Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe. |
| Protection of first-aiders | : pUC18 Control Plasmid DNA | Not applicable. |
| | 1.42 M 2-Mercaptoethanol | Not applicable. |
| | XL1-Blue Competent Cells | Not applicable. |
| Notes to physician | : No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled. | |

5. Fire-fighting measures

| | | |
|------------------------------------|--------------------------------|--|
| Flammability of the product | : pUC18 Control Plasmid DNA | Non-flammable. |
| | 1.42 M 2-Mercaptoethanol | Non-flammable. |
| | XL1-Blue Competent Cells | Non-flammable. |
| Products of combustion | : pUC18 Control Plasmid DNA | No specific data. |
| | 1.42 M 2-Mercaptoethanol | Decomposition products may include the following materials: carbon oxides sulfur oxides |
| | XL1-Blue Competent Cells | Decomposition products may include the following materials: carbon oxides sulfur oxides halogenated compounds metal oxide/oxides |

Extinguishing media

| | | |
|---|---|---|
| Suitable | : pUC18 Control Plasmid DNA | Use an extinguishing agent suitable for the surrounding fire. |
| | 1.42 M 2-Mercaptoethanol | Use an extinguishing agent suitable for the surrounding fire. |
| | XL1-Blue Competent Cells | Use an extinguishing agent suitable for the surrounding fire. |
| Not suitable | : pUC18 Control Plasmid DNA | Not applicable. |
| | 1.42 M 2-Mercaptoethanol | Not applicable. |
| | XL1-Blue Competent Cells | Not applicable. |
| Special protective equipment for fire-fighters | : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode. | |
| Special remarks on fire hazards | : pUC18 Control Plasmid DNA | Not available. |
| | 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent Cells | Not available. |
| Special remarks on explosion hazards | : Not available. | |

6 . Accidental release measures

| | | |
|----------------------------------|-----------------------------|--|
| Personal precautions | : pUC18 Control Plasmid DNA | No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8). |
| | 1.42 M 2-Mercaptoethanol | No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8). |
| | XL1-Blue Competent Cells | No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8). |
| Environmental precautions | : pUC18 Control Plasmid DNA | Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air). |
| | 1.42 M 2-Mercaptoethanol | Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air). |
| | XL1-Blue Competent Cells | Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air). |
| Methods for cleaning up | | |
| Small spill | : pUC18 Control Plasmid DNA | Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor. |
| | 1.42 M 2-Mercaptoethanol | Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor. |
| | XL1-Blue Competent Cells | Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor. |

7 . Handling and storage

| | | |
|-----------------|-----------------------------|--|
| Handling | : pUC18 Control Plasmid DNA | Wash thoroughly after handling. |
| | 1.42 M 2-Mercaptoethanol | Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling. |
| | XL1-Blue Competent Cells | Do not ingest. Wash thoroughly after handling. |

7. Handling and storage

Storage : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8. Exposure controls/personal protection

Product name

Exposure limits

United States

1.42 M 2-Mercaptoethanol
2-Mercaptoethanol

AIHA WEEL (United States, 1/2008).
TWA: 0.2 ppm 8 hour(s).

XL1-Blue Competent Cells
Glycerol

ACGIH TLV (United States, 1/2008).
TWA: 10 mg/m³ 8 hour(s). Form: Mist
OSHA PEL (United States, 11/2006).
TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
TWA: 15 mg/m³ 8 hour(s). Form: Total dust
OSHA PEL 1989 (United States, 3/1989).
TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
TWA: 10 mg/m³ 8 hour(s). Form: Total dust

Manganese dichloride

ACGIH TLV (United States, 1/2008).
TWA: 0.2 mg/m³, (as Mn) 8 hour(s).
OSHA PEL 1989 (United States, 3/1989).
CEIL: 5 mg/m³, (as Mn)
NIOSH REL (United States, 12/2001).
TWA: 1 mg/m³, (as Mn) 10 hour(s).
STEL: 3 mg/m³, (as Mn) 15 minute(s).
OSHA PEL (United States, 11/2006).
CEIL: 5 mg/m³, (as Mn)

Sucrose

ACGIH TLV (United States, 1/2008).
TWA: 10 mg/m³ 8 hour(s).
OSHA PEL 1989 (United States, 3/1989).
TWA: 15 mg/m³ 8 hour(s). Form: Total dust
TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
NIOSH REL (United States, 12/2001).
TWA: 10 mg/m³ 10 hour(s). Form: Total
TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction
OSHA PEL (United States, 11/2006).
TWA: 15 mg/m³ 8 hour(s). Form: Total dust
TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction

Dimethyl sulfoxide

AIHA WEEL (United States, 1/2008).
TWA: 250 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

8 . Exposure controls/personal protection

| | |
|-------------------------|---|
| Skin | : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing. |
| Respiratory | : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator. |
| Hands | : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. |
| Other protection | : Not available. |
| Hygiene measures | : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location. |

9 . Physical and chemical properties

| | |
|-----------------------------------|--|
| Physical state | : pUC18 Control Plasmid Liquid. DNA |
| | : 1.42 M 2-Mercaptoethanol Liquid. |
| | : XL1-Blue Competent Liquid. Cells |
| Flash point | : pUC18 Control Plasmid Not applicable. DNA |
| | : 1.42 M 2-Mercaptoethanol Not applicable. |
| | : XL1-Blue Competent Not applicable. Cells |
| Color | : pUC18 Control Plasmid Not available. DNA |
| | : 1.42 M 2-Mercaptoethanol Not available. |
| | : XL1-Blue Competent Not available. Cells |
| Odor | : pUC18 Control Plasmid Not available. DNA |
| | : 1.42 M 2-Mercaptoethanol Not available. |
| | : XL1-Blue Competent Not available. Cells |
| pH | : pUC18 Control Plasmid Neutral. DNA |
| | : 1.42 M 2-Mercaptoethanol Neutral. |
| | : XL1-Blue Competent Neutral. Cells |
| Boiling/condensation point | : pUC18 Control Plasmid Lowest known value: 100°C (212°F) (Water). DNA |
| | : 1.42 M 2-Mercaptoethanol Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F) |
| | : XL1-Blue Competent Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F) Cells |
| Melting/freezing point | : pUC18 Control Plasmid May start to solidify at the following temperature: 0°C (32°F) DNA This is based on data for the following ingredient: Water. |
| | : 1.42 M 2-Mercaptoethanol May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water. |
| | : XL1-Blue Competent May start to solidify at the following temperature: 19.8°C Cells (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 3.02°C (37.4°F) |

9 . Physical and chemical properties

| | | |
|-------------------------|--------------------------|---|
| Relative density | : pUC18 Control Plasmid | Not available. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Only known value: 1.1 (Water = 1) (2-Mercaptoethanol). |
| | XL1-Blue Competent Cells | Weighted average: 1.29 (Water = 1) |
| Vapor pressure | : pUC18 Control Plasmid | Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) |
| | DNA | (Water). |
| | 1.42 M 2-Mercaptoethanol | Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C) |
| | XL1-Blue Competent Cells | Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.11 kPa (15.83 mm Hg) (at 20°C) |
| Vapor density | : pUC18 Control Plasmid | Highest known value: 0.62 (Air = 1) (Water). |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1) |
| | XL1-Blue Competent Cells | Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.98 (Air = 1) |
| Evaporation rate | : pUC18 Control Plasmid | Not available. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent Cells | 0.026 (Dimethyl sulfoxide) compared with Butyl acetate. |

10 . Stability and reactivity

| | |
|--|---|
| Stability and reactivity | : The product is stable. |
| Incompatibility with various substances | : Highly reactive or incompatible with the following materials: oxidizing materials and organic materials. Reactive or incompatible with the following materials: acids. |
| Hazardous decomposition products | : pUC18 Control Plasmid Under normal conditions of storage and use, hazardous decomposition products should not be produced. DNA 1.42 M 2-Mercaptoethanol Under normal conditions of storage and use, hazardous decomposition products should not be produced. XL1-Blue Competent Cells Under normal conditions of storage and use, hazardous decomposition products should not be produced. |
| Conditions of reactivity - Flammability | : Flammable in the presence of the following materials or conditions: open flames, sparks and static discharge. |

11 . Toxicological information

Acute toxicity

| Product/ingredient name | Result | Species | Dose | Exposure |
|-------------------------|-------------|---------|-------------|----------|
| Dimethyl sulfoxide | LD50 Dermal | Rat | 40 gm/kg | - |
| | LD50 Oral | Rat | 14500 mg/kg | - |
| Sucrose | LD50 Oral | Rat | 29700 mg/kg | - |
| Manganese dichloride | LD50 Oral | Rat | 250 mg/kg | - |
| Glycerol | LD50 Dermal | Rabbit | >10 gm/kg | - |
| | LD50 Oral | Rat | 12600 mg/kg | - |
| Potassium chloride | LD50 Oral | Rat | 2600 mg/kg | - |

| | | |
|-------------|--------------------------|---|
| Eyes | : pUC18 Control Plasmid | No known significant effects or critical hazards. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Irritating to eyes. |
| | XL1-Blue Competent Cells | No known significant effects or critical hazards. |

11 . Toxicological information

| | | |
|------------|--------------------------|--|
| Skin | : pUC18 Control Plasmid | No known significant effects or critical hazards. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Irritating to skin. May cause sensitization by skin contact. |
| Inhalation | XL1-Blue Competent | No known significant effects or critical hazards. |
| | Cells | |
| | | |
| Ingestion | : pUC18 Control Plasmid | No known significant effects or critical hazards. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Toxic if swallowed. |
| | XL1-Blue Competent | Toxic if swallowed. |
| | Cells | |

Classification

| Product/ingredient name | ACGIH | IARC | EPA | NIOSH | NTP | OSHA |
|--------------------------|-------|------|-----|-------|-----|------|
| XL1-Blue Competent Cells | | | | | | |
| Sucrose | A4 | - | - | - | - | - |

Potential chronic health effects

| | |
|-----------------------|---|
| Chronic effects | : Contains material that may cause target organ damage, based on animal data. |
| Carcinogenicity | : No known significant effects or critical hazards. |
| Mutagenicity | : No known significant effects or critical hazards. |
| Teratogenicity | : No known significant effects or critical hazards. |
| Developmental effects | : No known significant effects or critical hazards. |
| Fertility effects | : No known significant effects or critical hazards. |

Over-exposure signs/symptoms

| | | |
|-----------------------|--------------------------|---|
| Inhalation | : No specific data. | |
| Ingestion | : No specific data. | |
| Skin | : No specific data. | |
| Eyes | : No specific data. | |
| Target organs | : pUC18 Control Plasmid | Not available. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent | Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea. |
| | Cells | |
| Other adverse effects | : pUC18 Control Plasmid | Not available. |
| | DNA | |
| | 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent | Not available. |
| | Cells | |

12 . Ecological information

| | |
|-----------------------|---|
| Environmental effects | : No known significant effects or critical hazards. |
|-----------------------|---|

12 . Ecological information

Aquatic ecotoxicity

| Product/ingredient name | Test | Result | Species | Exposure |
|-------------------------|------|--------------------------------------|---------|----------|
| Dimethyl sulfoxide | - | Acute LC50 35 to 37 ml/L Fresh water | Fish | 96 hours |
| | - | Acute LC50 34000000 ug/L Fresh water | Fish | 96 hours |
| Manganese dichloride | - | Acute EC50 4700 ug/L Fresh water | Daphnia | 48 hours |
| Glycerol | - | Acute LC50 54 to 57 ml/L Fresh water | Fish | 96 hours |
| Potassium chloride | - | Acute EC50 83000 ug/L Fresh water | Daphnia | 48 hours |
| | - | Acute LC50 337 mg/L Fresh water | Daphnia | 48 hours |
| | - | Acute LC50 435000 ug/L Fresh water | Fish | 96 hours |

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

| | | |
|--------------------|-----------------------------|---|
| HCS Classification | : pUC18 Control Plasmid DNA | Not regulated. |
| | 1.42 M 2-Mercaptoethanol | Toxic material Irritating material Sensitizing material |
| | XL1-Blue Competent Cells | Toxic material Target organ effects |

15 . Regulatory information

| | | |
|--------------------------|---|--|
| | pUC18 Control Plasmid DNA | Not available. |
| | 1.42 M 2-Mercaptoethanol | Not available. |
| | XL1-Blue Competent Cells | Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea. |
| U.S. Federal regulations | : pUC18 Control Plasmid DNA | United States inventory (TSCA 8b): All components are listed or exempted. |
| | 1.42 M 2-Mercaptoethanol | United States inventory (TSCA 8b): All components are listed or exempted. |
| | XL1-Blue Competent Cells | United States inventory (TSCA 8b): All components are listed or exempted. |
| | pUC18 Control Plasmid DNA | SARA 302/304/311/312 extremely hazardous substances: No products were found. SARA 302/304 emergency planning and notification: No products were found. SARA 302/304/311/312 hazardous chemicals: No products were found. SARA 311/312 MSDS distribution - chemical inventory - hazard identification: No products were found. |
| | 1.42 M 2-Mercaptoethanol | SARA 302/304/311/312 extremely hazardous substances: No products were found. SARA 302/304 emergency planning and notification: No products were found. SARA 302/304/311/312 hazardous chemicals: 2-Mercaptoethanol SARA 311/312 MSDS distribution - chemical inventory - hazard identification: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard |
| | XL1-Blue Competent Cells | SARA 302/304/311/312 extremely hazardous substances: No products were found. SARA 302/304 emergency planning and notification: No products were found. SARA 302/304/311/312 hazardous chemicals: Potassium chloride; Glycerol; Manganese dichloride; Sucrose; Dimethyl sulfoxide SARA 311/312 MSDS distribution - chemical inventory - hazard identification: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard; Dimethyl sulfoxide: Immediate (acute) health hazard, Delayed (chronic) health hazard |
| | pUC18 Control Plasmid DNA | Clean Water Act (CWA) 307: No products were found. |
| | 1.42 M 2-Mercaptoethanol | Clean Water Act (CWA) 307: No products were found. |
| | XL1-Blue Competent Cells | Clean Water Act (CWA) 307: No products were found. |
| | pUC18 Control Plasmid DNA | Clean Water Act (CWA) 311: Edetic acid |
| 1.42 M 2-Mercaptoethanol | Clean Water Act (CWA) 311: No products were found. | |
| XL1-Blue Competent Cells | Clean Water Act (CWA) 311: No products were found. | |

15 . Regulatory information

| | |
|------------------------------|--|
| pUC18 Control Plasmid DNA | Clean Air Act (CAA) 112 accidental release prevention: No products were found. |
| 1.42 M 2-Mercaptoethanol | Clean Air Act (CAA) 112 accidental release prevention: No products were found. |
| XL1-Blue Competent Cells | Clean Air Act (CAA) 112 accidental release prevention: No products were found. |
| pUC18 Control Plasmid DNA | Clean Air Act (CAA) 112 regulated flammable substances : No products were found. |
| 1.42 M 2-Mercaptoethanol | Clean Air Act (CAA) 112 regulated flammable substances : No products were found. |
| XL1-Blue Competent Cells | Clean Air Act (CAA) 112 regulated flammable substances : No products were found. |
| pUC18 Control Plasmid DNA | Clean Air Act (CAA) 112 regulated toxic substances: No products were found. |
| 1.42 M 2-Mercaptoethanol | Clean Air Act (CAA) 112 regulated toxic substances: No products were found. |
| XL1-Blue Competent Cells | Clean Air Act (CAA) 112 regulated toxic substances: No products were found. |

SARA 313

| | <u>Product name</u> | <u>CAS number</u> | <u>Concentration</u> |
|--|---------------------------------|-------------------|----------------------|
| Form R - Reporting requirements | XL1-Blue Competent Cells | | |
| | Manganese dichloride | 7773-01-5 | 5 - 10 |
| | Hexaammincobalt trichloride | 10534-89-1 | 0.1 - 1 |
| Supplier notification | XL1-Blue Competent Cells | | |
| | Manganese dichloride | 7773-01-5 | 5 - 10 |
| | Hexaammincobalt trichloride | 10534-89-1 | 0.1 - 1 |

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

| | | |
|--------------------------|--------------------------------|---|
| State regulations | : pUC18 Control Plasmid DNA | <p>Connecticut Carcinogen Reporting: None of the components are listed.</p> <p>Connecticut Hazardous Material Survey: None of the components are listed.</p> <p>Florida substances: None of the components are listed.</p> <p>Illinois Chemical Safety Act: None of the components are listed.</p> <p>Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.</p> <p>Louisiana Reporting: None of the components are listed.</p> <p>Louisiana Spill: None of the components are listed.</p> <p>Massachusetts Spill: None of the components are listed.</p> <p>Massachusetts Substances: None of the components are listed.</p> <p>Michigan Critical Material: None of the components are listed.</p> <p>Minnesota Hazardous Substances: None of the components are listed.</p> <p>New Jersey Hazardous Substances: None of the components are listed.</p> <p>New Jersey Spill: None of the components are listed.</p> <p>New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.</p> <p>New York Acutely Hazardous Substances: None of the components are listed.</p> <p>New York Toxic Chemical Release Reporting: None of the components are listed.</p> <p>Pennsylvania RTK Hazardous Substances: None of the components are listed.</p> |
|--------------------------|--------------------------------|---|

15 . Regulatory information

Rhode Island Hazardous Substances: None of the components are listed.

1.42 M 2-Mercaptoethanol **Connecticut Carcinogen Reporting:** None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: 2-Mercaptoethanol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: 2-Mercaptoethanol
Rhode Island Hazardous Substances: None of the components are listed.

XL1-Blue Competent Cells

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol; Sucrose
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: The following components are listed: Manganese dichloride
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.

15 . Regulatory information

Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride; Sucrose

Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

| | | | |
|--------------------|---|---------------------------|--|
| Label requirements | : | pUC18 Control Plasmid DNA | NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED. |
| | | 1.42 M 2-Mercaptoethanol | HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION. |
| | | XL1-Blue Competent Cells | HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA. |

Date of issue : 01/09/2009

Version : 1

Notice to reader

DISCLAIMER: This Material Safety Data Sheet is offered without charge to the clients of Agilent Technologies. Data is the most current available to Agilent Technologies at the time of preparation and is issued as a matter of information only, no warranty as to its accuracy or completeness is expressed or implied.

Indicates information that has changed from previously issued version.

1. PRODUCT AND COMPANY INFORMATION

INVTITROGEN CORPORATION
 1600 FARADAY AVE.
 CARLSBAD, CA 92008
 760/603-7200

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

INVTITROGEN CORPORATION
 3 FOUNTAIN DR.
 INCHINNAN BUSINESS PARK
 PAISLEY, PA4 9RF
 SCOTLAND
 44-141 814-6100

INVTITROGEN CORPORATION
 P.O. BOX 12-502
 PENROSE
 AUCKLAND 1135
 NEW ZEALAND
 64-9-579-3024

INVTITROGEN CORPORATION
 2270 INDUSTRIAL ST.
 BURLINGTON, ONT
 CANADA L7P 1A1
 905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES) : 301/431-8585 (24 HOUR)
 800/451-8346 (24 HOUR)
 NON-EMERGENCY INFORMATION: 800/955-6288

Product Name: LIBRARY EFFICIENCY DHEALPHA COMPETENT CELLS
 Stock Number: 18263012

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
 These products are for laboratory research use only and are not intended for human or animal diagnostics, therapeutic, or other clinical uses.

Synonyms:
 Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

| | | |
|--------------------|---------|---------|
| Component | CAS No. | Percent |
| DIMETHYL SULFOXIDE | 67-68-5 | 3 - 7 |

3. HAZARDS IDENTIFICATION

***** EMERGENCY OVERVIEW *****
Warning!
Irritant:
Harmful if absorbed

Potential Health Effects:
Eye: Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin: Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage. Upon prolonged or repeated exposure, harmful if absorbed through the skin. May cause minor systemic damage.

Inhalation: Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache. No toxicity expected from inhalation.

Ingestion: Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

Chronic: No data on cancer.

4. FIRST AID MEASURES

Eye: Flush eyes with plenty of water for at least 20 minutes, retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin: Wash with soap and water. Get medical attention if irritation develops or persists.

Inhalation: Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion: Do not induce vomiting and seek medical attention immediately. Drink two

MATERIAL SAFETY DATA SHEET
 LIBRARY EFFICIENCY DHSALPHA COMPETENT CELLS
 NITROGEN CORPORATION
 MSDS ID: 18263

Page 3 of 8
 Revised 9/30/03
 Replaces 9/05/03
 Printed 9/30/03

4. FIRST AID MEASURES (CONT.)

glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
 Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:

Not available.

Upper Flammable Limit %:

Not available.

Lower Flammable Limit %:

Not available.

Autoignition Temperature Deg C:

Not available.

Extinguishing Media:

Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. DMSO undergoes a violent exothermic reaction 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, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen peroxide, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Firefighting Techniques/Equipment:

Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:

Carbon dioxide Carbon monoxide Sulfur containing gases

5. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

| | | | |
|---|--|----------|---------|
| <u>MATERIAL SAFETY DATA SHEET</u> | | Page | 4 OF 8 |
| LIBRARY EFFICIENCY DHSALPHA COMPETENT CELLS | | Revised | 9/30/03 |
| INVIITROGEN CORPORATION | | Replaces | 9/05/03 |
| MSDS ID: 18263 | | Printed | 9/30/03 |

6. ACCIDENTAL RELEASE MEASURES (CONT.)

Spill Cleanup:
 Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill.
 Ventilate the contaminated area.
 Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
 Ambient

Handling Procedures:
 Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
 Keep closed or covered when not in use.

Storage Procedures:
 Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
 Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

| | | |
|--------------------|---------------------------|---------------------------|
| Component | OSHA PEL | AGCIH TWA |
| DIMETHYL SULFOXIDE | (ppm) Not established. | (ppm) Not established. |

Engineering Controls:
 Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
 Safety glasses should be the minimum eye protection.
 Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash

MATERIAL SAFETY DATA SHEET

| | |
|----------|---------|
| Page | 6 of 8 |
| Revised | 9/30/03 |
| Replaces | 9/05/03 |
| Printed | 9/30/03 |

LIBRARY EFFICIENCY DIALPHA COMPETENT CELLS
NITROGEN CORPORATION
MSDS ID: 19253

10. STABILITY AND REACTIVITY (CONT.)

degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Sulfur containing gases.

Hazardous Polymerization: Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG

Target Organs: Blood. Eyes. Skin.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. ECOLOGICAL INFORMATION

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades slowly.

Cell Line Designation: 293 (HEK293)**ATCC® Catalog No. CRL-1573™****Table of Contents:**

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description**Organism:** *Homo sapiens* (human)**Tissue:** kidney; transformed with adenovirus 5 DNA**Age:** fetus**Morphology:** epithelial**Growth properties:** adherent**Doubling time:** about 19 hours**Tumorigenic:** tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells.**Receptors expressed:** vitronectin**Virus susceptibility:** human adenoviruses**DNA profile (STR analysis)**

Amelogenin: X
 CSF1PO: 11,12
 D13S317: 12,14
 D16S539: 9,13
 D5S818: 8,9
 D7S820: 11,12
 TH01: 7,9.3
 TPOX: 11
 vWA: 16,19

Depositors: F.L. Graham**Comments:** Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome, it is now clear that only left end sequences are present. The line is excellent for titrating human adenoviruses.

The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37C.

The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit.

The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2).

Karyotype: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %.

The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired.

There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.**Purified DNA:** from this line is available as ATCC Catalog No. CRL-1573D™ (10 µg).**Biosafety Level: 2**Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bml4/bml4toc.htm.**Use Restrictions****These cells are distributed for research purposes only.**

293 cells, their products, or their derivatives may not be distributed to third parties. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.*It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this*

point on should be carried out under strict aseptic conditions.

- Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to 7 minutes.
- Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
- Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37°C.

- Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
- If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
- If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Add 2.0 to 3.0 ml of 0.25% (w/v) Trypsin-0.53mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 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.

- Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
- Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10³ (3) to 6 X 10³ (3) viable cells/cm² is recommended.
Subcultivation Ratio; 1:10 to 1:20 weekly.
- Incubate cultures at 37°C.
- Subculture when cell concentration is between 6 and 7 X 10⁴ cells/cm²

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

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Product Information Sheet for ATCC® CRL-1573™

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ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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01/09

Cell Line Designation: U-2 OS
ATCC® Catalog No. HTB-96

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)

Tissue: osteosarcoma; bone

Age: 15 years

Gender: female

Ethnicity: Caucasian

Morphology: epithelial

Growth properties: adherent

DNA profile (STR analysis):

Amelogenin: X
 CSF1PO: 13
 D13S317: 13
 D16S539: 11,12
 D5S818: 11
 D7S820: 11,12
 TH01: 6,9,3
 TPOX: 11,12
 vWA: 14,18

AntigenExp: Blood Type A; Rh+; HLA A2, Aw30, B12, Bw35, B40(+/-)

Products: osteosarcoma derived growth factor (ODGF)

Receptors expressed: insulin-like growth factor I (IGF-I); insulin-like growth factor II (IGF-II)

Depositors: Hellstrom

Comments: J. Ponten and E. Saksela derived this line (originally 2T) in 1964 from a moderately differentiated sarcoma of the tibia of a 15 year old girl. Viruses were not detected during co cultivation with WI-38 cells or in CF tests against SV40, RSV or adenoviruses. Mycoplasma contamination was detected and eliminated in 1972.

Karyotype: Cell line U-2 OS is chromosomally highly altered, with chromosome counts in the hypertriploid range. We did not find the hypodiploid cell population described by J. Ponten, et al. [PubMed: 68131797]. Instead, most of the population has slightly higher counts than first described. Very few normal chromosomes are present, but a high number of stable marker chromosomes are identified. Different chromosomal rearrangements involving the same chromosomes (N1, N7, N9, and N11 particularly), are seen. Twenty-two markers are found including: t(9qter-->9q21::1p36-->1p::?), 7p+, iso(17q), t(15q:?), 4q+, del(3)(q21), 5q(aberrant) and others.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., 1995) the ATCC manual on quality control (Hay et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bml4/bml4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

- Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium, and spin at approximately 125 xg for 5 to 7 minutes.
- Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio), and dispense into a 25 cm² or a 75 cm² culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
- Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

- Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
- If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
- If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.
- Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

- Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
- Add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: 1:3 to 1:6
- Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

2 to 3 times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

- (additional references are available in the catalog at www.atcc.org)
- Heldin CH et al. A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. *Nature* 319: 511, 1986 PubMed: 86118705
- Ponten J and Saksela E. Two established in vitro cell lines from human mesenchymal tumours. *Int. J. Cancer* 2: 434-447, 1967 PubMed: 68131797
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- Landers JE et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a

stabilized wild-type p53 protein. *Cancer Res.* 57: 3562-3568. 1997 PubMed: 97413643

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Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition. ASM press, Washington, DC.

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Immortalization of Endothelial Cells

Endothelial cells are critical components of wound healing, inflammation, circulation, and tumor growth metastases. Endothelial cells are difficult to isolate and culture. A unique approach has been used to immortalize endothelial cells, which are more amenable to culture. The cell line, designated HMEC-1, provides a ready source of human endothelial cells for research, including studies on the physiologic and pathologic factors that induce endothelial mitosis, pharmacologic studies for the screening of various agents, and toxicologic studies for the cosmetic and pharmaceutical industry.

Inventors: Edwin W Ades, Thomas Lawley, and Francisco J Candal

CDC Reference Number: E-036-91/0

USPTO Serial Number: none

Patent Number:

RESEARCH AND EVALUATION MATERIALS TRANSFER AGREEMENT (REMTA)

This Agreement is made and entered into as of this 10th day of December, 2009 by and between the Centers for Disease Control and Prevention (CDC),

with principal address located at 1600 Clifton Road, N.E., Atlanta, Georgia 30333 (hereinafter referred to as "CDC") and

The University of Western Ontario

a non-profit (university or institution) with principal address located at

1151 Richmond Street, London, Ontario, Canada, N6A 3K7

(hereinafter referred to as "Recipient").

Recipient, through its below identified Scientist (hereinafter "Recipient's Scientist"), has requested that CDC, through its below identified Scientist (hereinafter "CDC's Scientist") provide Recipient the below described Original Material. Recipient's Scientist shall use the Material for Recipient's Research Project as described below.

Recipient's Scientist: James R Hammond
Dept of Physiology and Pharmacology
M266 Medical Sciences Bldg
University of Western Ontario
London, Ontario N6A 5C1
Canada

CDC's Scientist: Merry Y. Liu, MPH
Centers for Disease Control and Prevention (CDC)
Mail Stop D-34
1600 Clifton Road, N.E.
Atlanta, Georgia 30333

Original Material: Human Microvascular Endothelial Cells (HMEC-1). HMEC-1 refers to a cell line resulting from the transfection of human dermal microvascular endothelial cells with a PBR-322 based plasmid containing the coding region for the simian virus 40 large T antigen. The cell line has been immortalized by Dr. Edwin Ades and Mr. Francisco J. Candal of CDC and Dr. Thomas Lawley, of Emory

University (hereinafter "Emory"). Emory and CDC have filed a patent application which claims the cell line and uses thereof. Emory has authorized the CDC to distribute HMEC-1 in accordance with this Agreement.

Recipient's Research Project:

Role of purine nucleoside and nucleobase transport and metabolism in the microvascular endothelial cell regulation of vascular function.

Adenosine is well established as a vasodilator acting via extracellular adenosine receptors located in the vasculature. Adenosine is released from cells under conditions of cellular stress and is taken back up into cells via a family of 'nucleoside transporters'. We are interested in how manipulation of purine metabolism in microvascular endothelial cells, including transport activity, impacts on the vasodilatory properties of adenosine as well as the production of deleterious oxygen and nitrogen free radical species. We wish to test whether the HMEC-1 cell line can be used as a model cell for these studies. We have conducted studies to date using primary microvascular endothelial cells, which are difficult and expensive to maintain. If the HMEC-1 cell line has similar nucleoside/nucleobase metabolism/transport characteristics to the primary cell lines, then we intend to extend these studies using the HMEC-1 cell line. These studies are directly relevant to therapeutic interventions for diabetes and for ischemia/reperfusion injury in heart failure and stroke.

For purposes of this Agreement, "Material" shall mean the above described Original Material plus any "Progeny" and "Unmodified Derivatives".

"Progeny" shall mean unmodified descendants from the Material, such as virus from virus, cell from cell, or organisms from organisms.

"Unmodified Derivatives" shall mean substances created by Recipient which constitute an important unmodified functional sub-unit or an expression product of the Original Material. Some examples include: subclones of unmodified cell lines; purified or fractionated sub-sets of the Original Material such as novel plasmids or vectors; proteins expressed by the Original Material; and DNA/RNA sequences for such expressed proteins.

"Modifications" shall mean substances created by Recipient which contain or incorporate the Material.

Article 1. Use of Material by Recipient

1.1 The Material is the property of CDC and Emory University and is to be used by Recipient solely for Recipient's Research Project at Recipient's institutional facilities only and only under the direction of Recipient's Scientist. Use for any commercial purpose, such as for screening, or production for sale is prohibited under

this Agreement. Recipient is specifically prohibited from using the Material during the course of any research sponsored by private concerns such as pharmaceutical or biotechnology companies. Emory and CDC are making the Material available under non-exclusive license agreements for commercial use through the NIH, Office of Technology Transfer, and Licensing Branch. If Recipient desires to make any commercial use of the Material, Recipient should contact the NIH, Licensing Branch or CDC, Technology Transfer Office. Neither CDC nor Emory makes any representation as to whether a commercial license is available or shall be granted to Recipient.

1.2 Recipient and Recipient's Scientist shall not transfer the Material to anyone who does not work under Recipient's Scientist's direct supervision at Recipient's facilities without the prior written consent of CDC. Recipient's Scientist shall refer any request for the Material to CDC's Scientist.

1.3 Recipient agrees in its use of the Material to comply with all applicable statutes, regulations and guidelines, including Public Health Service PHS regulations and guidelines. Recipient agrees not to use the Materials for research involving human subjects or clinical trials in the United States without complying with 21 CFR Part 50 and 45 CFR Part 46. Recipient agrees not to use the Materials for research involving human subjects or clinical trials outside of the United States without notifying CDC, in writing, of such research or trials and complying with the applicable regulations of the appropriate national control authorities. Written notification to CDC of research involving human subjects or clinical trials outside of the United States shall be given no later than sixty (60) days prior to commencement of such research or trials.

Article 2. Ownership and Use of Modifications

2.1 Recipient shall inform CDC's Scientist in writing of the creation of any Modifications within ninety (90) days of their creation.

2.2 Recipient shall own Modifications, provided that CDC and Emory shall retain ownership of any Material incorporated in such Modifications.

2.3 Recipient shall be free, at its discretion, to pursue legal protection for and commercialization of Modifications. However, Recipient recognizes that commercial use of Modifications may require a license from CDC and Emory and that neither CDC nor Emory shall have any obligation to grant such a license to Recipient or any third party.

2.4 Except as expressly provided under this Agreement, no rights are provided to Recipient under patents, patent applications, trade secrets or other proprietary rights of CDC or Emory.

Article 3. Acknowledgement of Use of Material

Recipient shall acknowledge Dr. Edwin Ades and Mr. Fransisco J. Candal of CDC and Dr. Thomas Lawley of Emory University as the developers of the Material in all oral presentations or written publications pertaining to any research conducted by Recipient using the Material in a mutually-accepted manner.

Article 4. Representations and Liability

4.1 RECIPIENT ACKNOWLEDGES THAT ANY MATERIAL DELIVERED TO IT UNDER THIS AGREEMENT IS EXPERIMENTAL IN NATURE. NEITHER EMORY NOR CDC MAKES ANY REPRESENTATIONS NOR EXTENDS ANY WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, WITH RESPECT TO THE MATERIAL. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, NOR DOES CDC OR EMORY REPRESENT THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADE SECRET, TRADEMARK OR OTHER RIGHTS OF THIRD PARTIES.

4.2 Recipient assumes all liability for damages which may arise from its use, storage or disposal of the Material. Neither CDC nor Emory shall be liable to Recipient for any loss, claim, or demand by Recipient, or made against Recipient by any other party, due to or arising from the use of the Material by Recipient. To the extent permitted by law, Recipient shall indemnify, defend and hold harmless, CDC and Emory, its trustees, assignees, agents and employees from any claim asserted against them arising from the negligence or willful misconduct in the use of the Materials or Modifications by Recipient or its agents or employees.

Article 5. Treatment of Confidential Information

Recipient shall treat in confidence, for a period of three (3) years from the date of its disclosure, any written information pertaining to the Material provided to Recipient by CDC or CDC's Scientist which is stamped "Confidential". Excluded from this obligation shall be any information that was previously known to Recipient or that is, or becomes publicly available during said three (3) year period or which is disclosed to Recipient without confidentiality obligations by a third party having the right to make such disclosure. This obligation of confidentiality shall not apply to any disclosure required by law, provided that Recipient shall notify CDC of any disclosure required by law in sufficient time to permit CDC or Emory to object, if CDC or Emory so desire.

Article 6. Termination

6.1 This Agreement shall terminate upon the earliest of the following events:

- (i) upon thirty (30) days' written notice by either party to the other; or

- (ii) upon completion of Recipient's Research Project; or
- (iii) when the Material becomes generally available to third parties, for example, through reagent catalogs or from a repository under the Budapest Treaty.

If termination shall occur under 6.1 (iii), Recipient shall be bound to CDC by the least restrictive terms applicable to the Material from then-available sources.

6.2 Recipient shall discontinue its use of the Material and shall, upon the written request of CDC, return or destroy any remaining material upon termination of this Agreement.

Article 7. Survival

Articles 3, 4, and 5 shall survive termination of this Agreement for any reason.

Article 8. Additional Requirements

- 8.1** FedEx # for Shipping: 1238-0320-5
- 8.2** For the described Material, the CDC requires a fee of \$327.00 (see attached User Fee Invoice).

Signatures Begin on the Next Page

Research and Evaluation Materials Transfer Agreement (REMTA)

Centers for Disease Control and Prevention (CDC)

and

University of Western Ontario

Agreed and Accepted for CDC:

By: _____

Date: _____

Agreed and Accepted for Recipient:

By: 

Date: Dec 13, 2009

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Product Description

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Cell Biology

ATCC® Number: **CRL-1663™** [Order this Item](#) Price: **\$407.00**

Designations: **UMR-108**

Depositors: AE Bogden

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Rattus norvegicus* (rat)

Morphology: fibroblast

Source: **Organ:** bone
Strain: Sprague-Dawley
Disease: osteosarcoma

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.

Receptors: parathyroid hormone (PTH); 1-25(OH)2D3 (bone resorbing steroid hormone)

Comments: The UMR-108 cell line is a clonal derivative of a transplantable rat osteosarcoma that had been induced by injection of radiophosphorous (32P). The cells are responsive to PTH, prostaglandins and bone resorbing steroids. The PTH responsiveness of UMR-106 is less than that of the related cell line UMR-106 (ATCC [CRL-1661](#)). Both the original sarcoma and the cloned line were developed by T.J. Martin at the University of Sheffield.

[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%.

[Subculturing:](#) **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week
Remove medium, add fresh 0.25% trypsin for 2 to 3 minutes, remove trypsin and let the culture stand at room temperature for 10 to 15 minutes. Add fresh medium, aspirate and dispense into new flasks.

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Cell Line Designation: C2C12

ATCC Catalog No. CRL-1772™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Mus musculus* (mouse)

Strain: C3H

Tissue: muscle; myoblast

Morphology: myoblast

Growth Properties: adherent

Depositor: B. Paterson

Comments: This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel.

The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins.

Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic.

Tested and found negative for ectromelia virus (mousepox).

Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture.

Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice

has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium, and spin at approximately 125 xg for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
5. Transfer the cell suspension to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
6. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence

of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Do not allow the culture to reach confluence. The myoblast component of the population will rapidly become depleted if the culture is allowed to reach confluence. Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels to give approximately $1.5 \times 10^5 - 1 \times 10^6$ cells/T75.

Subcultivation ratio: 1:4 to 1:10

6. Place culture vessels in incubators at 37°C.
- Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Every two or three days.

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%

This medium is formulated for use with a 5% CO₂ in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO₂ in air atmosphere is then recommended).

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Recommended Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog at www.atcc.org)

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Cell Biology

ATCC® Number:

CCL-33™

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Price:

\$355.00

Designations:

PK(15)

Depositors:

Cutter Laboratories, Inc.

Biosafety Level:

1

Shipped:

frozen

Medium & Serum:

[See Propagation](#)

Growth Properties:

adherent

Organism:

Sus scrofa (pig)

Morphology:

epithelial

Source:

Organ: kidney
Disease: normal

Cellular Products:

plasminogen activator; keratin

Permits/Forms:

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Applications:

transfection host ([Roche FuGENE® Transfection Reagents](#))

Virus Resistance:

poliovirus 2

Age:

adult

Comments:

The presence of a porcine papovavirus in PK(15) cells has been reported in cells obtained from multiple sources including the ATCC . [\[53398\]](#) [\[53399\]](#)

The Foreign Animal Disease Diagnostic Laboratory of the US Department of Agriculture has determined that ATCC CCL-33 is not infected with Hog cholera virus or African swine fever virus, and uses this line to screen for those viruses. [\[105588\]](#)

The cell line harbors an endogenous C-type retrovirus. [\[26185\]](#) [\[53399\]](#) [\[56104\]](#)

The cells are positive for porcine circovirus (PCV) antigens.

The cells are positive for keratin by immunoperoxidase staining.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Temperature: 37.0°C

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Subculturing: **Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: 2 to 3 times per week
Rinse the cell sheet 2 times with fresh 0.25% trypsin, 0.03% EDTA solution, remove trypsin and allow the culture to stand at room temperature for 5 to 10 minutes. Add fresh medium, aspirate and dispense into new flasks.

Preservation: culture medium 95%, DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):[ATCC 30-2003](#)
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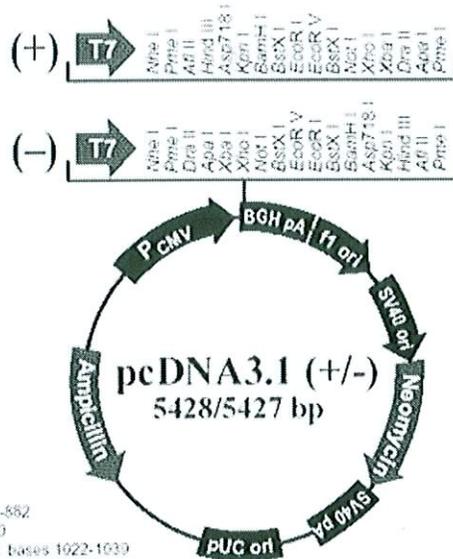
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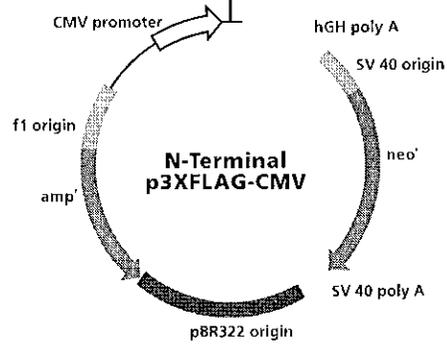
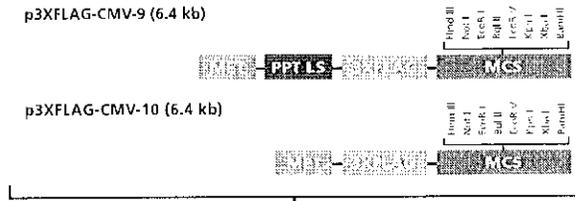
Comments for pcDNA3.1 (+)
5428 nucleotides

- CMV promoter: bases 232-819
- T7 promoter/primer site: bases 863-882
- Multiple cloning site: bases 895-1010
- pcDNA3.1-BGH reverse priming site: bases 1022-1039
- BGH polyadenylation sequence: bases 1026-1252
- H1 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 3617-4267 (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)

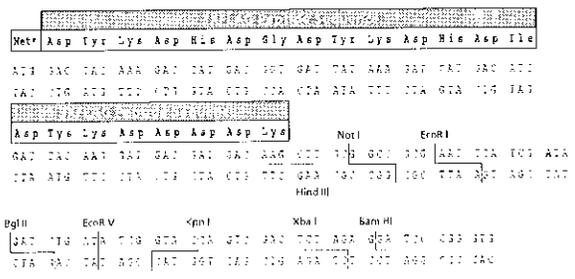
[pcDNA3.1^{\(-\)} Restriction map](#)

[pcDNA3.1^{\(+\)} Restriction map](#)

Plasmid(s)



Multiple Cloning Site
(p3XFLAG-CMV-9* and p3XFLAG-CMV-10)



*For pFLAG-CMV-9, the Met-preprotrypsin leader sequence (PPT LS) precedes the FLAG coding sequence.