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

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:

Name	UWO E-mail Address	Date of Biosafety Training
Derek Bone	derekbone@gmail.com	Sept 2004
Jamie Park	jpark44@uwo.ca	Sept 2007
Frances Cunningham	fcunnin2@uwo.ca	Sept 2008
Scott Hughes	shughe3@uwo.ca	June 2009
Samer Serhan	samy1000@gmail.com	Sept 2010
Diana Quinonez	dquinon@uwo.ca	Sept 2009
Samantha Li	sli288@uwo.ca	June 2010

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 inclubators, 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.

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.

The biological safety procedures enforced in the lab regarding mammalian cell culture are summarized below:

- Proper lab attire such as lab coats, gloves and eye protection are used regularly.
- A class II biological safety cabinet is used for all mammalian cell culture manipulations. The cabinet is maintained on an annual basis.
- Surface areas are disinfected with 70% ethanol.
- All biohazardous waste is disposed of in a clearly marked biohazard bag/container which is autoclaved for decontamination; autoclaved waste is subsequently labeled as treated biomedical waste and disposed of by custodial staff.
- All glassware containing biohazardous material is allowed to disinfect in bleach before washing and autoclaving.
- Spills are handled by bleaching and covering the spilled material with paper towels, using appropriate safety protection. Towels are then place in a biohazard bag for autoclaving.
- Upon completion of mammalian cell manipulations, gloves are disposed of and hands are washed.
- All pipettes utilized are sterile plugged cotton pipettes.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES ✓ **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	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	0.1 litre	Stratagene	O 1 O 2 O 2+ O 3
DH5alpha	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	0.1 litre	Invitrogen	O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			

*Please attach a Material Safety Data Sheet or equivalent f

E. coli - Level 1

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	✓ **Yes** O No	Heart (purchased from Lonza)	
Rodent	✓ **Yes** O No	Microvascular endothelial cells from mouse skeletal muscle	2009-030
Non-human primate	O Yes ✓ **No**		
Other (specify)	O Yes ✓ **No**		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	✓ **Yes** O No	HEK293 U2_OS HMEC-1	2 1 1	ATCC ATCC CDC
Rodent	✓ **Yes** O No	UMR108 C2C12	1 1	ATCC ATCC
Non-human primate	O Yes ✓ **No**			
Other (specify)	✓ **Yes** O 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 O 1 ✓ **2** O 2+ O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms
1.0 and 2.0? ✓ **YES** O NO If no, pleas

4.2 ⇒ Yes
⇒ E. coli

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
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?
O 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 O YES, please specify _____ ⊗NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ⊗NO
- SV 40 Large T antigen O YES ⊗NO
- E1A oncogene O YES ⊗NO
- Known oncogenes O YES, please specify _____ ⊗NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ⊗NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

6.1 Will live animals be used? O **YES** O 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 O YES, specify: _____ ⊗NO

6.5 Will the agent(s) be shed by the animal: O YES O 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)? O YES O **No** If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

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

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

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

_____

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin_____

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

8.5 How much of the toxin is stored*? _____

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

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

9.0 Insects

9.1 Do you use insects? O YES O **NO** If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____

_____

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

_____

_____

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

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

_____

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

_____

_____

_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

11.0 Import Requirements

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

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE _____

13.0 Containment Levels

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

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

⊙ **YES, permit # if on-campus: _BIO-UWO-0195__**
O NO, please certify
O 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 _____ 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:

Lonza Walkersville Inc.
8830 Biggs Ford Road
Walkersville, MD 21793 0107
Tel (301) 898 7025
Fax (301) 845 4024

Lonza

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 Min.	Max.	Results
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

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

0004639339

Packing List

0004639339

Shipping Point:	Walkersville 21793-0127		
Delivery Date:	29-Jul-2010	**Shipping Terms:**	FCA - Free carrier
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 Item	Product Code/ 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 Walkersville, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Walkersville, MD 21793-0127 USA

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-I, 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.

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

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 DNA 200231-42 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

MSDS'

2. Hazards identification

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		XL1-Blue Competent Cells	Liquid.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
OSHA/HCS status	:	pUC18 Control Plasmid DNA	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.
		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 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.

2 . Hazards identification

	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	1.42 M 2-Mercaptoethanol	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	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.
Routes of entry :	pUC18 Control Plasmid DNA	Eye contact. Ingestion.
	1.42 M 2-Mercaptoethanol	Dermal contact. Eye contact. Inhalation. Ingestion.
	XL1-Blue Competent Cells	Eye contact. Inhalation. Ingestion.

Potential acute health effects

Eyes :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to eyes.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Skin :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed.
	XL1-Blue Competent Cells	Toxic if swallowed.
Medical conditions aggravated by over-exposure :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	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.
	XL1-Blue Competent Cells	Repeated or prolonged exposure to the substance can produce target organs damage.
Over-exposure signs/symptoms :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
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 DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		XL1-Blue Competent Cells	Liquid.
Flash point	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Color	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
pH	:	pUC18 Control Plasmid DNA	Neutral.
		1.42 M 2-Mercaptoethanol	Neutral.
		XL1-Blue Competent Cells	Neutral.
Boiling/condensation point	:	pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
		1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
		XL1-Blue Competent Cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F)
Melting/freezing point	:	pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) 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 Cells	May start to solidify at the following temperature: 19.8°C (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 DNA	Not available.
		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 DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (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 DNA	Highest known value: 0.62 (Air = 1) (Water).
		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 DNA	Not available.
		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 DNA: Under normal conditions of storage and use, hazardous decomposition products should not be produced. 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 DNA	No known significant effects or critical hazards.
		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 DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
		XL1-Blue Competent Cells	Toxic if swallowed.

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

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

	Product name	CAS number	Concentration
Form R - Reporting requirements :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt 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

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: None of the components are listed.
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: None of the components are listed.

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.**

MATERIAL SAFETY DATA SHEET

Revised 9/30/03
Replaces 9/05/03
Printed 9/30/03

LIBRARY EFFICIENCY DH5ALPHA COMPETENT CELLS
INVITROGEN CORPORATION
MSDS ID: 18263

1. PRODUCT AND COMPANY INFORMATION

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

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

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

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

INVITROGEN 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 DH5ALPHA 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, therpeutic, 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

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 periodate, 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

6. 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.

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 (ppm)	AGCIH TWA (ppm)
DIMETHYL SULFOXIDE	Not established.	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

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Gloves should be used as minimum hand protection.

Respiratory:
Use supplied-air respiratory equipment as required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalies. 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 periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO

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.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
DIMETHYL SULFOXIDE	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES: None assigned.

PRODUCT SAFETY PHRASES: Not applicable.

PRODUCT CLASSIFICATION:

15. REGULATORY INFORMATION (CONT.)

Not classified

Component	EINECS Number
DIMETHYL SULFOXIDE	200-664-3

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

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

ATCC®

Product Information

Info for Cell Line(s)

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

Table of Contents:

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 seqment 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/bmbl4/bmbl4toc.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*

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to7 minutes.

4. 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 cm^2 or a 75 cm^2 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).*

5. 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.
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.

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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. 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.

3. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

4. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm^2 is recommended.
Subcultivation Ratio; 1:10 to 1:20 weekly.

5. Incubate cultures at 37°C.

6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm^2

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% CO_2 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.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

References

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

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

Table of Contents:

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: osteosacoma derived growth factor (ODGF)
Receptors expressed: insulin-like growth factor I (IGF-I); insulin-like growth factor II (IGF-I)
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/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.*

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3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 or a 75 cm^2 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).*

5. 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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 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. Add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: 1:3 to 1:6

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% CO_2 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

Raile K et al. **Human osteosarcoma (U-2 OS) cells express both insulin-like growth factor-I (IGF-I) receptors and insulin-like growth factor-II/mannose-6- phosphate (IGF-II/M6P) receptors and synthesize IGF-II: autocrine growth stimulation by IGF-II via the IGF-I receptor.** J. Cell. Physiol. 159: 531-541, 1994 PubMed: 94245830

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

Moradpour D et al. **Characterization of cell lines allowing titghtly regulated expression of heapatitis C virus core protein.** Virology 222: 51-63. 1996 PubMed: 96400111

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992). **ATCC Quality Control Methods for Cell Lines.** 2nd edition. Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods **11**:223-227. 1988.

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Centers for Disease Control (1993). **Biosafety in Microbiological and Biomedical Laboratories** Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services: 3rd Edition U.S. Government Printing Office Washington D.C.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

07/07

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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:

This page last reviewed November 13, 2003

Centers for Disease Control and Prevention

Office of Director
Technology Transfer Office

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: D. Hanks Date: Dec. 10, 2009

Reviewed By NM

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Cell Biology

ATCC® Number:	CRL-1663™ Order this Item
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.

Price: $407.00

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References:

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Cell Line Designation: C2C12
ATCC Catalog No. CRL-1772™

Table of Contents:

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 to7 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

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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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 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 cm^2 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 x 10^5 – 1 x 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% CO_2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 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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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.

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Product Description

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Cell Biology

ATCC® Number: CCL-33™ Order this Item

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

Applications: transfection host (Roche FuGENE® Transfection Reagents)

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

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

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
recommended serum:ATCC 30-2020

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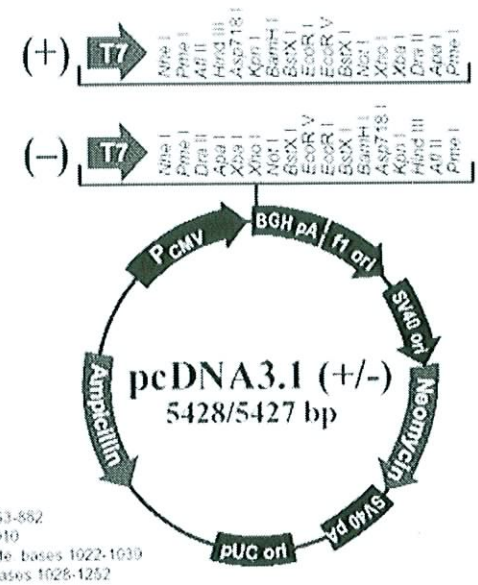


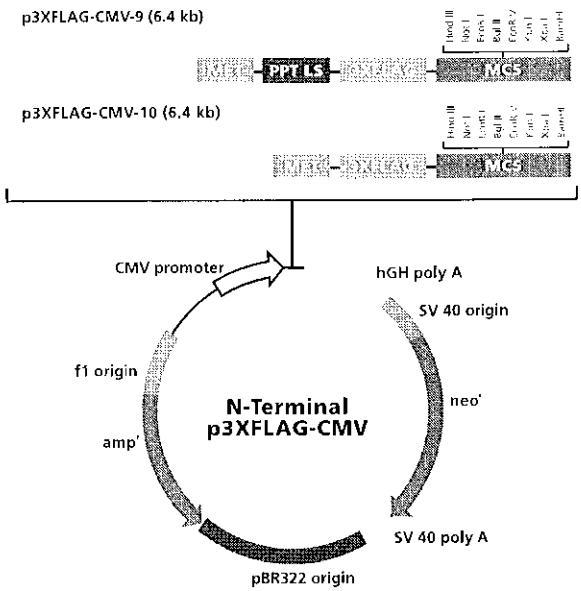
Comments for pcDNA3.1 (+)
5428 nucleotides

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

pcDNA3.1(-) Restriction map

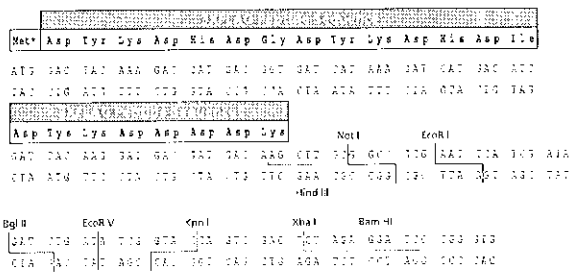
pcDNA3.1(+) Restriction map

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.