THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: April 9, 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 biohazardous 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 biohazards 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 Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Dr David O'Gorman / Dr. Bing Siang Gan
DEPARTMENT	Biochemistry
ADDRESS	268 Grosvenor St, London, ON Rm EA-137
PHONE NUMBER	x 64397
EMERGENCY PHONE NUMBER(S)	519-471-6457 / x 66097
EMAIL	dogorman@uwo.ca / bsgan@rogers.com

Location of experimental work to be carried out: Building(s) Lawson Health Research Institute Room(s) F1-104

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR / Plastic Surgery Education Foundation
GRANT TITLE(S): Molecular Mechanisms of Dupuytren's Disease
Molecular Mechanisms of Abnormal Scarring

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Brett Thurlow
Justin Crawford
Christina Raykha
Andrew Gould

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

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)? INVITROGEN
Please describe the risk (if any) of escape and how this will be mitigated:
No risk (non-pathogenic E. coli)

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli (DH5α)	O Yes ☑ No	O Yes ☑ No	O Yes ☑ No	0.1 L	Invitrogen	☑ 1 O 2 O 2+ O 3
~~Lentivirus~~ (virus-like particle deficient)	~~O Yes ☑ No~~ O Yes O No	~~O Yes ☑ No~~ O Yes O No	~~O Yes ☑ No~~ O Yes O No	~~0.05 L~~	~~Invitrogen~~	~~O 1 ☑ 2 O 2+ O 3~~ O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☑ 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	Surgically resected tissue	Not applicable
Rodent	O Yes ☑ No		
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)*	Supplier / Source
Human	☑ Yes ○ No	HaCaT	CLS - Germany
Rodent	☑ Yes ○ No	NIH 3T3	ATCC - USA
Non-human primate	○ Yes ☑ No		
Other (specify)	○ Yes ☑ No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		○ Yes ○ No ○ Unknown		○ 1 ○ 2 ○ 2+ ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No ○ Unknown		○ 1 ○ 2 ○ 2+ ○ 3
Human Organs or Tissues (unpreserved)	Surgically resected tissue (in-house surgeries)	○ Yes ☑ No ☑ Unknown	—	○ 1 ☑ 2 ○ 2+ ○ 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α	pCR 2.1	Invitrogen	POSTN	No phenotypic change, gene not expressed. Bacteria used for plasmid amplification. Bacteria will gain antibiotic resistance for ampicillin + Kanamycin

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

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

4.3 Will genetic modification(s) involving viral vectors be made? ✓ YES, complete table below ✗ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Lentivirus	plenti/V5	Invitrogen	POSTN	~~enhancement~~ constitutive expression

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV ~~✓ YES, please specify gp160, Rev~~ ✗ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ~~✓ YES, specify V5~~ ✗ 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? ✓ YES O NO

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

4.7 Will this be expected to increase the containment level required? ✓ 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 ✓ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

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

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

_____

_____

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

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 ⊘ No If no, please proceed to section 8.0

7.2 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 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 O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

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

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

8.3 What is the 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 ⊘ 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:
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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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 O 2 Ø 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
Ø YES, permit # if on-campus BIO – LHRI – 0052 re-certification 17/08/2010
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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: 20 July 2010

14.2 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

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:
Individual will report to OH+S, STHC for care + follow-up.

15.0 Approvals

1) UWO Biohazard 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: August 12/2010

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

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

To: Biohazards Subcommittee, UWO

From: Dr. David O'Gorman, Lawson Health Research Institute

19 January 2011

To Whom It May Concern:

Enclosed is a modification of our current Biohazardous Agents Registry Form, initially submitted in July 2010.

In the time this form has been under review, new information as to the effectiveness of the Invitrogen Virapower Lentiviral system has come to light, and on the basis of that information we no longer intend to use the system as was planned when the initial submission of the Form was drafted.

At this time we have no firm plans as to an approach that would take the place of the Virapower system, and so we have no replacement information to offer. On the instruction of the UWO Biosafety Coordinator, we have redacted reference to the lentiviral work on the enclosed pages and are including this short memo as explanation of our reasoning for doing so.

David B. O'Gorman, M.Sc., Ph.D.
Assistant Professor
Departments of Biochemistry and Surgery
University of Western Ontario

Description of Work

In brief, our work revolves around the culture of primary tissue explants for the purpose of generating human cells capable of acting as proxy models for a disease state of interest (specifically Dupuytren's Contracture and / or abnormal scarring phenotypes).

We pursue this research through the CL-2 approved culture of tissue explants from surgical resections (done in-house at the Hand and Upper Limb Clinic, St. Joseph's Health Care) and subsequent manipulations of those cultured cells in downstream applications typical of molecular biology research. All samples received from the surgical team have been screened by the referring physician for known infectious diseases and are flagged as such. Regardless, all samples received in the lab are treated as potentially containing infectious materials and are handled with appropriate precautions.

For applications and investigations not suited to the use of primary cell cultures, we also maintain immortalized cell lines (HaCaT keratinocytes and NIH 3T3 fibroblasts) obtained from commercial sources. All cell cultures are stored in approved liquid nitrogen-containing cell storage units when not in active culture

In all cases, cell culture takes place entirely within an approved and inspected CL-2 facility, is carried out only by trained individuals, and is maintained in accordance with published biosafety protocols. Any waste generated is decontaminated with 10% (final ~~volume~~ concentration) bleach prior to disposal in the institutional biohazard waste stream. Virtually all equipment used in cell culture is disposable by nature, and is treated as any other waste as required.

In addition to the use of primary cell cultures, basic molecular investigation in the lab requires the use of standard practices of gene manipulation, including the use of non-pathogenic E. coli bacterial cultures for the purpose of amplification and manipulation of various genetic sequences of interest. Such cultures are commercially obtained and cultured in accordance with standard protocols. Waste or spills generated are decontaminated with 10% (final volume) bleach prior to disposal in the institutional biohazard waste stream. Equipment and glassware used in such cultures is decontaminated with bleach, washed and re-sterilised on-site.

Finally, though not taking place in the lab currently, research requirements dictate the generation of genetically modified organisms through viral transduction of primary cells (at some point in the near future). Such approaches are required when using primary culture due to the demonstrated inefficiency of plasmid transfection.

The current intention is to use a commercially available system for generation of a replication defective lentiviral vector containing our gene(s) of interest. This system (ViraPower from Invitrogen) is already in use in the institute, and our lab technician (Andrew Gould) has experience with the system and with the necessary safety protocols required for operating in a CL-2 environment with CL-3 precautions (previously established, in consultation with the on-site Safety Officer, as the required level of protection for work with this system).

Waste generated from this future objective will be disposed of in accordance with established protocols -- bleach decontamination of all waste prior to removal from the culture hood, followed by immediate autoclave sterilization prior to disposal in the institutional biohazard waste stream.

MATERIAL SAFETY DATA SHEET

Page	1 of 8
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

MSDS,

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.

invitrogen

Material Safety Data Sheet

Revision Date: 03-Mar-2006

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

Product code 351275
Product name VIRAPOWER PKG. MIX 195 UG, LYOPHILIZED

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

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

Form
Solid

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Sensitization	No information available
Target Organ Effects	No information available

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Solid

Important Health Safety and Environmental Information

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

Water solubility	No data available

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

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End of Safety Data Sheet

HaCaT

Cell name	Description	Order no.	Units	Price, Euro
HaCaT	Human keratinocyte cell line	300493	cryovial	430.00
HaCaT	Human keratinocyte cell line	330493	vital	490.00

P. Boukamp

Boukamp P. et al. (3.7 MB)
Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line

Designation:	HaCaT
Depositor:	DKFZ, Heidelberg
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age/Stage:	62 years
Gender:	male
Tissue:	Skin
Celltype:	keratinocyte
Growth Properties:	monolayer
Description:	in vitro spontaneously transformed keratinocytes from histologically normal skin
Culture Medium	DMEM medium (high glucose) supplemented with 2 mM L-glutamine and 10% fetal calf serum.
Subculturing:	Remove medium, rinse with 0.05% EDTA, add 0.05% EDTA solution and incubate for 10 min at 37°C. Take off EDTA, add fresh 0.05% trypsin/0.025% EDTA solution (final concentrations) and let culture sit at 37°C until the cells detach (approx. 5 minutes). Add fresh medium, aspirate and dispense into new flasks.
Split Ratio:	A ratio of 1:5 to 1:10 is recommended
Fluid Renewal:	2 times weekly
Freeze Medium	CM-1 (CLS - Cell Lines Service)
Sterility:	Tests for mycoplasma, bacteria and fungi were negative
Biosafety Level:	1
Tumorigenic:	no
Karyotype:	Aneuploid (hypotetraploid)

Cell line(s)

References:

Boukamp P, Dzarlieva-Petrusevska RT, Breitkreuz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol. 106: 761-771, 1988.
Boukamp P, Popp S, Altmeyer S, Hulsen A, Fasching C, Cremer T, Fusenig NE. Sustained nontumorigenic phenotype correlates with a largely stable chromosome content during long-term culture of the human keratinocyte line HaCaT. Genes, Chromosomes and Cancer 19: 201-214, 1997.

ATCC®

Product Information Sheet for ATCC CRL-1658™

Cell Line Designation: NIH/ 3T3
ATCC Catalog No. CRL-1658™

Table of Contents:

Cell Line Description

Organism: *Mus musculus* (mouse)
Strain: NIH/Swiss
Tissue: embryo
Morphology: fibroblast
Growth properties: adherent
VirusSuscept: murine sarcoma viruses; murine leukemia viruses
Depositors: S.A. Aaronson
Comments: The NIH/3T3, a continuous cell line of highly contact-inhibited cells was established from NIH Swiss mouse embryo cultures in the same manner as the original random bred 3T3 (ATCC CCL-92™) and the inbred BALB/c 3T3 (ATCC CCL-163™). The established NIH/3T3 line was subjected to more than 5 serial cycles of subcloning in order to develop a subclone with morphologic characteristics best suited for transformation assays. These cells are useful for DNA transfection and transformation studies.
Tested and found negative for ectromelia virus (mousepox).

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 growth medium and spin at approximately 125 xg for 5 to 7 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.

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

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Or contact your local distributor.

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

Never allow the culture to become completely confluent. Subculture at 80% confluency or less.
Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 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.

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 supension to new culture vessels. Use 3-5 x 10(3) cells/cm^2 and subculture about every 3 days.

Note: In order to maintain this property of **high contact inhibition** it is necessary to transfer routinely at only high dilutions, otherwise variants tend to be selected having reduced contact inhibition. Such low density make culture vessels appear sparse and cell growth sensitive to sub-optimal temperature and media conditions.

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

Two times per week.

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:

- bovine calf 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).
The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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 are available in the catalog at www.atcc.org)

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Loffler S et al. **CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus.** J. Virol. 71: 42-49, 1997 PubMed: 97138295

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

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

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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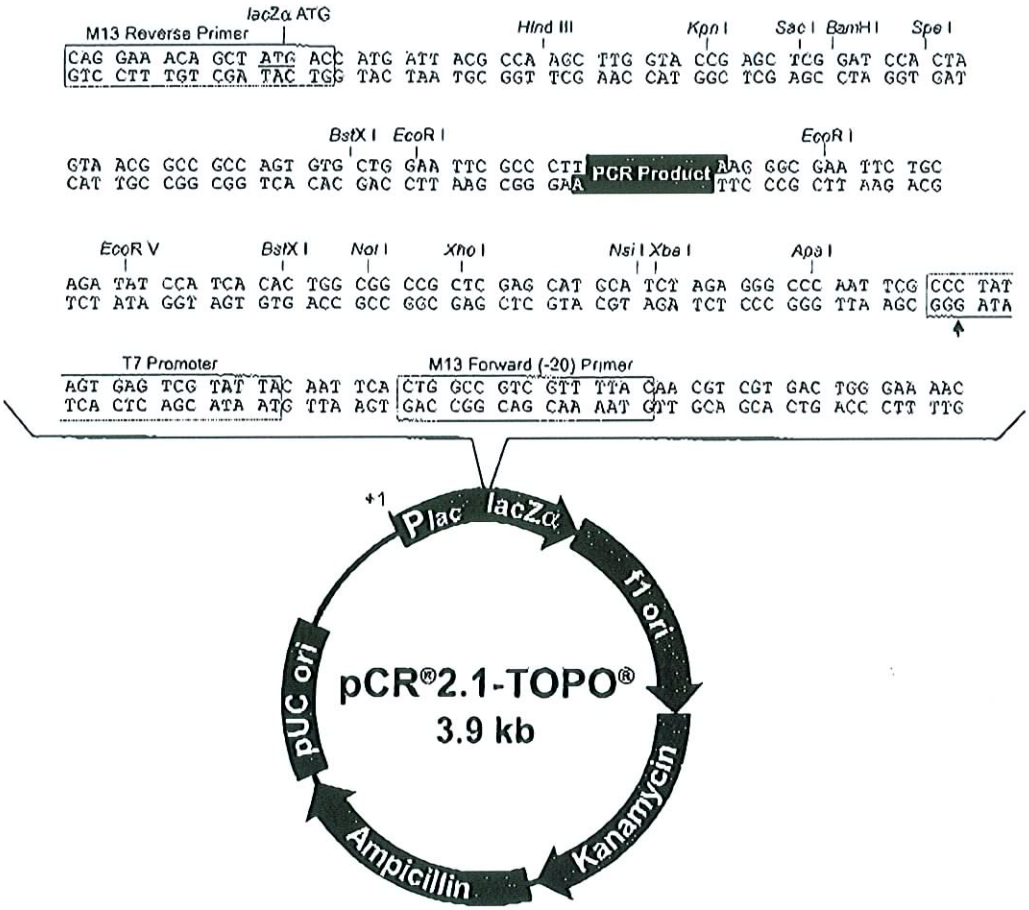

Comments for pCR®2.1-TOPO®
3931 nucleotides

*Lac*Zα fragment: bases 1-547
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-357
T7 promoter/priming site: bases 364-383
M13 Forward (-20) priming site: bases 391-406
f1 origin: bases 548-985
Kanamycin resistance ORF: bases 1319-2113
Ampicillin resistance ORF: bases 2131-2991
pUC origin: bases 3136-3809

Info on Plasmid(s)

Invitrogen™
life technologies