Modification Form for Permit BIO-UWO-0256

Permit Holder: Lakshman Gunaratnam

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

Ola Ismail

Xinghong Xhang

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E.coli, Lentivirus	
Approved Primary and Established Cells	Mouse[primary] kidney, spleen, blood, bone marrow. Human[established]786-0, 769-P, HEK293, HEK293T/17, Jurkat, HK-2 Rodent[established] JAWSII, mIMCD-3, CMT-93 Porcine [established]LLC-PK1, canine	DC, 2, 4 (mouse)
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[plasmids]: pcDNA3. [vectors]: pLUX-puro proprietory	
Approved Use of Animals	Musculus	
Approved Biological Toxin(s)	C3 Exotoxin, Phalloidin	SB202190 (S)-(+)-Camptothecin GM6001 ~~4-Bromo-2-phenylimidazole~~ dihydrochloride DMSO

DMSO does not need to be listed (per march meeting)

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

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Current Classification: 2+ **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Jun 29, 2010

Date of Last Modification (if applicable): Feb 08, 2011

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

1) Captothecin: Used to induce apoptosis in cells. Apoptotic cells are used as targets for phagocytosis in our experiments.[1]
2) SB 202190: Used to block MEK kinase in our cells. MEK kinase is believed to involved in metalloproease-mediated cleavage of KIM-1.[2]
3) GM6001: Used to block metalloproteases involved in KIM-1 shedding.[2] GM6001 will be injected into mice after ischemia-reperfusion injury surgery. This protocol will be submitted as a modification to our animal protocol.
4) DC 2.4 mouse dendritic cells were acquired from Dr. Kenneth Rock at Dana Farber Cancer Institute (via MTA). They will be used to study the interrelationship of antigen presenting cells with our kidney epithelial cells.

1. Ichimura T, Asseldonk EJ, Humphreys BD, Gunaratnam L, Duffield JS, Bonventre JV. Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. *J Clin Invest.* May 2008;118(5):1657-1668.
2. Zhang Z, Humphreys BD, Bonventre JV. Shedding of the urinary biomarker kidney injury molecule-1 (KIM-1) is regulated by MAP kinases and juxtamembrane region. *J Am Soc Nephrol.* Oct 2007;18(10):2704-2714.

For the Captothecin:
How much (in ug) do you handle at once? 5mM in 15 uL
How much (in ug) do you store? 100mg
Please provide an LD50. Acute oral toxicity (LD50): 50.1 mg/kg [Mouse]

per email March 23, 2011

For the SB202190:
How much (in ug) do you handle at once? 0.05mg
How much (in ug) do you store? 5mg
Please provide an LD50. Acute dermal toxicity (LD50): 40000 mg/kg [Rat].

For the GM6001:
How much (in ug) do you handle at once? 0.010.05 mg
How much (in ug) do you store? 1mg-1g
Please provide an LD50. LD50: Not available.

0.01-.05 mg per e-mail March 23, 2011

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	SB202190
Proposed Use Dose:	50 µg
Proposed Storage Dose:	500 µg
LD_{50} (species):	40000 µg

Calculation:

40000 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 2000000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 200000

Comments/Recommendations:

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Captothecin
Proposed Use Dose:	1.7 µg
Proposed Storage Dose:	10000 µg
LD_{50} (species):	50100 µg

Calculation:

50100 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 2505000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 250500

Comments/Recommendations:

Material Safety Data Sheet

Version 4.1
Revision Date 10/22/2010
Print Date 02/07/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : (*S*)-(+)-Camptothecin

Product Number : C9911
Brand : Sigma
Product Use : For laboratory research purposes.

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

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

Telephone : +19058299500
Fax : +19058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

D1B Toxic Material Causing Immediate and Serious Toxic Effects — Toxic by ingestion

GHS Classification

Acute toxicity, Oral (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H301	Toxic if swallowed.

Precautionary statement(s)

P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P321	Specific treatment (see supplemental first aid instructions on this label).
P330	Rinse mouth.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

HMIS Classification

Health hazard:	2
Flammability:	0
Physical hazards:	0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.

Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	Toxic if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Formula : $C_{20}H_{16}N_2O_4$
Molecular Weight : 348.35 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
(S)-(+)-Camptothecin			
7689-03-4	-	-	-

4. FIRST AID MEASURES

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

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

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

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

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

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

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

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

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

7. HANDLING AND STORAGE

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

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

Recommended storage temperature: 2 - 8 °C

Keep in a dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

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

Eye protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	no data available

Safety data

pH	no data available
Melting/freezing point	Melting point/range: 260 °C (500 °F) - dec.
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available

Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 153 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

Genotoxicity in vitro - mouse - lymphocyte
Other mutation test systems

Genotoxicity in vitro - Hamster - ovary
Sister chromatid exchange

Genotoxicity in vitro - Human - leukocyte
DNA inhibition

Genotoxicity in vitro - Hamster - Lungs
Mutation in mammalian somatic cells.

Genotoxicity in vitro - Hamster - Lungs

Sister chromatid exchange

Genotoxicity in vitro - mouse - leukocyte
DNA inhibition

Genotoxicity in vitro - Hamster - ovary
DNA damage

Genotoxicity in vitro - Human - HeLa cell
DNA inhibition

Genotoxicity in vitro - Chicken - Embryo
DNA inhibition

Genotoxicity in vitro - Human - HeLa cell
Other mutation test systems

Genotoxicity in vitro - Human - leukocyte
Other mutation test systems

Genotoxicity in vitro - Human - lymphocyte
Sister chromatid exchange

Genotoxicity in vitro - Hamster - Lungs
Cytogenetic analysis

Genotoxicity in vitro - Hamster - ovary
Cytogenetic analysis

Genotoxicity in vitro - Hamster - Lungs
DNA damage

Genotoxicity in vitro - mouse - lymphocyte
DNA inhibition

Genotoxicity in vitro - mouse - leukocyte
DNA damage

Genotoxicity in vitro - Monkey - Kidney
DNA damage

Genotoxicity in vitro - Human - lymphocyte
Cytogenetic analysis

Genotoxicity in vitro - Human - Other cell types
Cytogenetic analysis

Genotoxicity in vitro - Human - fibroblast
DNA damage

Genotoxicity in vitro - Human - HeLa cell
DNA damage

Genotoxicity in vitro - Human - lymphocyte
DNA damage

Genotoxicity in vitro - Human - Other cell types
DNA damage

Genotoxicity in vivo - mouse - Intraperitoneal
Cytogenetic analysis

Carcinogenicity

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

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

Reproductive toxicity

no data available

Teratogenicity

no data available

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

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

Aspiration hazard
no data available

Potential health effects

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

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: UQ0492000

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 1544 Class: 6.1 Packing group: III
Proper shipping name: Alkaloids, solid, n.o.s. ((S)-(+)-Camptothecin)
Marine pollutant: No

Poison Inhalation Hazard: No

IMDG
UN-Number: 1544 Class: 6.1 Packing group: III EMS-No: F-A, S-A
Proper shipping name: ALKALOIDS, SOLID, N.O.S. ((S)-(+)-Camptothecin)
Marine pollutant: No

IATA
UN-Number: 1544 Class: 6.1 Packing group: III
Proper shipping name: Alkaloids, solid, n.o.s. ((S)-(+)-Camptothecin)

15. REGULATORY INFORMATION

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

	CAS-No.
(S)-(+)-Camptothecin	7689-03-4

WHMIS Classification

D1B	Toxic Material Causing Immediate and Serious Toxic Effects	Toxic by ingestion

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

16. OTHER INFORMATION

Further information

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

Conforms to 93/112/EC and ISO 11014-1

SAFETY DATA SHEET

CALBIOCHEM®

Order Number **Customer Number**

1. Identification of the substance/preparation and of the company/undertaking

Product name : GM6001 in Solution

Chemical formula : $C_{20}H_{28}N_4O_4$

Synonym : Not available.

Catalog # : 364206

Supplier : EMD Biosciences, Inc.
10394 Pacific Center Court
San Diego, CA 92121
(858)450-5558/(800)854-3417
FAX: (858)453-3552

Emergency telephone number : Call Chemtrec®
(800)424-9300 (within U.S.A.)
(703)527-3887 (outside U.S.A.)

2. Composition / information on ingredients

Substance/Preparation : Substance

Chemical name*	CAS No.	EC Number	Symbol	R-Phrases
GM6001 in Solution		Not available.	Xi	R36/38

3. Hazards identification

Physical/chemical hazards : Not applicable.

Human health hazards : CAUTION!
MAY CAUSE EYE AND SKIN IRRITATION.

4. First-aid measures

First-Aid measures

Inhalation : If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Ingestion : Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin Contact : In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Eye Contact : Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Effects and symptoms

Skin Contact : Hazardous in case of skin contact (irritant). Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

Eye Contact : Hazardous in case of eye contact (irritant).

Aggravating conditions : Repeated or prolonged exposure is not known to aggravate medical condition.

5. Fire-fighting measures

Flammability of the Product : May be combustible at high temperature.

Extinguishing Media

Suitable : SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Hazardous thermal (de)composition products : These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...).

Special fire-fighting procedures : Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.

Protection of fire-fighters : Be sure to use an approved/certified respirator or equivalent.

6. Accidental release measures

Personal precautions	:	Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Small Spill and Leak	:	Absorb with an inert material and put the spilled material in an appropriate waste disposal
Large Spill and Leak	:	Absorb with an inert material and put the spilled material in an appropriate waste disposal.

7. Handling and storage

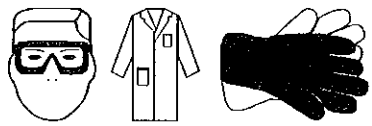
Handling	:	Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. If you feel unwell, seek medical attention and show the label when possible. Avoid contact with skin and eyes
Storage	:	Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 4°C (39.2°F)
Packaging materials		
Recommended use	:	Use original container.

8. Exposure controls/personal protection

Engineering measures	:	Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.
Hygiene measures	:	Wash hands after handling compounds and before eating, smoking, using lavatory, and at the end of day.

Ingredient Name	Occupational Exposure Limits
GM6001 in Solution	Not available.

Personal protective equipment

Skin and body	:	Lab coat.
Hands	:	Gloves.
Eyes	:	Splash goggles.
Protective Clothing (Pictograms)	:	

9. Physical and chemical properties

Physical state	:	Liquid. (Supplied in DMSO)
Color	:	Not available.
Molecular Weight	:	388.5 g/mole
Solubility	:	Not available.
Flash point	:	Not available.
Explosive properties	:	Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

10. Stability and reactivity

Stability	:	The product is stable.
Conditions to avoid	:	Hygroscopic; keep container tightly closed.
Hazardous Decomposition Products	:	These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...).

11. Toxicological information

RTECS #	:	**Not available.**
Local effects		
Skin irritation	:	Hazardous in case of skin contact (irritant).
Acute toxicity	:	LD50: Not available. LC50: Not available.
Chronic toxicity	:	Repeated or prolonged exposure is not known to aggravate medical condition.
Other Toxic Effects on Humans	:	Not available. Hazardous in case of skin contact (irritant), of eye contact (irritant). Not available.

To the best of our knowledge, the toxicological properties of this product have not been thoroughly investigated

Carcinogenic effects	:	Not available
Mutagenic effects	:	Not available.
Reproduction toxicity	:	Not available
Teratogenic effects	:	Not available.

12. Ecological information

Ecotoxicity	:	Not available.
Toxicity of the Products of Biodegradation	:	The product itself and its products of degradation are not toxic.

13. Disposal considerations

Methods of disposal: Waste of residues: Contaminated packaging	:	Waste must be disposed of in accordance with federal, state and local environmental control regulations.

14. Transport information

International transport regulations

Land - Road/Railway

ADR/RID Class	:	Not controlled under ADR (Europe).

Sea

IMDG Class	:	Not controlled under IMDG.

Air

IATA-DGR Class	:	Not controlled under IATA.
Special Provisions for Transport		Not applicable.

15. Regulatory information

EU Regulations

Hazard symbol(s)	:	
Classification	:	Irritant
Risk Phrases	:	R36/38- Irritating to eyes and skin.
Safety Phrases	:	S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
U.S. Federal Regulations	:	TSCA: No products were found. 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. SARA 313 toxic chemical notification and release reporting: No products were found. Clean Water Act (CWA) 307: No products were found. Clean Water Act (CWA) 311: No products were found. Clean air act (CAA) 112 accidental release prevention: No products were found. Clean air act (CAA) 112 regulated flammable substances: No products were found. Clean air act (CAA) 112 regulated toxic substances: No products were found.
HCS Classification	:	CLASS: Irritating substance.
State Regulations	:	
WHMIS (Canada)	:	Not controlled under WHMIS (Canada). No products were found.

16. Other information

Hazardous Material Information System (U.S.A.)

[illegible]	2
[illegible]	1
Reactivity	0
Personal Protection	J

National Fire Protection Association (U.S.A.)

Notice to Reader

Material Safety Data Sheet

Version 4.1
Revision Date 10/21/2010
Print Date 02/07/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	SB 202190
Product Number	:	S7067
Brand	:	Sigma
Product Use	:	For laboratory research purposes.

Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer	: Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+19058299500		
Fax	:	+19058299292		
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

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

GHS Classification

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

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/ eye protection/ face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P312	Call a POISON CENTER or doctor/ physician if you feel unwell.
P321	Specific treatment (see supplemental first aid instructions on this label).
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
P362	Take off contaminated clothing and wash before reuse.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

HMIS Classification

Health hazard:	2
Flammability:	0
Physical hazards:	0

Potential Health Effects

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

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 4-(4-Fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)-1H-imidazole

Formula : $C_{20}H_{14}FN_3O$

Molecular Weight : 331.34 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
SB 202190			
152121-30-7	-	-	-

4. FIRST AID MEASURES

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

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

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

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

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

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

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

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

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen fluoride

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

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

Environmental precautions
Do not let product enter drains.

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

7. HANDLING AND STORAGE

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

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

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

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

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

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

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

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

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available

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

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen fluoride

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity
no data available

Carcinogenicity

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

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

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
Inhalation - May cause respiratory irritation.

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

Aspiration hazard
no data available

Potential health effects

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

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

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

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

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

	CAS-No.
SB 202190	152121-30-7

WHMIS Classification

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

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

16. OTHER INFORMATION

Further information

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

DC 2.4

Cloned Dendritic Cells Can Present Exogenous Antigens on Both MHC Class I and Class II Molecules[1]

Zhenhai Shen,*‡ Glen Reznikoff,† Glenn Dranoff,† and Kenneth L. Rock[2]*‡

Pathways for presenting proteins from the extracellular fluids on MHC class I molecules have been described in macrophages. However, it is uncertain whether similar mechanisms exist in dendritic cells, because conventional preparations of these cells can be contaminated with macrophages. We addressed this issue by transducing granulocyte-macrophage CSF into bone marrow cultures followed by supertransfection with *myc* and *raf* oncogenes. These immortalized clones displayed dendritic morphology, and many expressed the dendritic cell-specific markers DEC-205 and 33D1 as well as high levels of MHC molecules and costimulatory molecules. Using these cloned dendritic cells, we found that exogenous OVA could be presented on both their MHC class I and class II molecules. This presentation was markedly enhanced when the Ag was particulate and internalized by phagocytosis. Presentation of particulate OVA on MHC class I molecules was insensitive to the weak base chloroquine, but was blocked by peptide aldehyde inhibitors of the proteasome, indicating that the class I-presented peptides were generated in the cytosol. Brefeldin A, which inhibits the exocytosis of newly synthesized proteins from the endoplasmic reticulum, also inhibited Ag presentation. These results establish that dendritic cells can present exogenous Ags on MHC class I molecules and appear to use a similar phagosome to cytosol pathway as macrophages. Therefore, dendritic cells are likely to play an important role in generating immune responses to tissue transplants and tumors in vivo. Furthermore, these findings provide an approach for targeting vaccine Ags into these cells to prime immune responses in vivo. *The Journal of Immunology,* **1997, 158: 2723–2730.**

MHC class I molecules play an important role in immune surveillance by displaying antigenic peptides on the cell surface (1). The majority of these presented peptides are generated by a multicatalytic proteolytic particle, the proteasome, which is present in the cytosol and nucleus of all eukaryotic cells (2). These antigenic fragments are then translocated into the endoplasmic reticulum (ER)[3] by the transporter associated with Ag presentation (TAP), where they bind to newly assembled MHC class I molecules and then are transported to the plasma membrane (1). As a consequence of these mechanisms, class I-presented peptides are derived in most situations exclusively from cellular and viral proteins synthesized by the APCs. Ags in the extracellular fluids do not gain access to this pathway in most cells and fail to be presented (3).

However, a subset of APCs can present exogenous Ags on class I molecules (4, 5), and this process is markedly enhanced when the Ag is internalized by phagocytosis (6, 7) or macropinocytosis (8). In these cells the exogenous Ag is transferred from the endocytic compartment into the cytosol where it is degraded and presented by the classical MHC class I pathway (8–10). Alternatively, in some cases peptides from the exogenous Ag appear to be generated in the endocytic compartment and to bind to MHC class I molecules on the cell surface (7, 11–13).

The APCs that can present exogenous Ags on MHC class I are quantitatively recovered from lymphoid tissues in a low density fraction that is enriched in macrophages and dendritic cells (DC) (5). That macrophages can mediate this form of Ag presentation has been shown in assays with highly purified macrophages and cloned macrophage cell lines (14). It has been more difficult to address whether DC possess a similar Ag-presenting capability because purified preparations of these cells are often contaminated with macrophages. Therefore, this question can only be resolved with absolutely pure DC. This is an important issue because DC are extremely potent APCs that are believed to play a key role in the initiation of T cell responses (15). This was the rationale in the present study for isolating cloned dendritic cell lines.

The exogenous pathway is active in vivo (6, 16) and probably plays an important role in generating CTL responses in several situations. A major pathway for stimulating CTL to tumors and transplanted tissue involves the representation of tumor or alloantigens on host bone marrow-derived APCs (17–19). Moreover, a similar pathway may be involved in generating anti-viral responses (3). Finally, this pathway can be exploited for stimulating T cell responses to Ags in vaccines (20). Therefore, it is important to elucidate the underlying cellular and molecular mechanisms underlying this pathway of presentation.

Materials and Methods

Cell lines and Abs

NIH J2 Leuk was provided by Dr. U. Rapp (Wurzburg, Germany) (21). FDCP-1 is a GM-CSF-dependent cell line used to measure GM-CSF production (22). The retrovirus-producing cell line Crip MFG-murine GM-CSF (23), the macrophage cell line A3.1A7 (14), and T-T hybridomas RF33.70 (anti-OVA, K^b) (24) and MF2.2D9 (anti-OVA$^+$ IA^b) (6) were

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[2] Address correspondence and reprint requests to Dr. Kenneth L. Rock, Division of Lymphocyte Biology, Dana Farber Cancer Institute, 44 Binney St., Boston, MA 02115.

[3] Abbreviations used in this paper: ER, endoplasmic reticulum; TAP, transporter associated with antigen presentation; DC, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; RT-PCR, reverse transcription-polymerase chain reaction; LLnL, *N*-acetyl-L-leucinyl-L-leucinal-L-norleucinal; LLM, *N*-acetyl-L-leucinyl-L-leucinyl-methional.

0022-1767/97/$02.00

previously described: mAbs Y3 (anti-H2-K^b) (25), Y3P (anti-IA) (26), 2.4G2 (anti-FcγRII) (27), 16-10A1 (anti-B7-1) (28), GL-1 (anti-B7-2) (29), YN1/1.7.4 (anti-ICAM-1) (30), D7 (anti-Ly6A/E) (31), M5/49 (anti-Thy-1) (32), M1/70 (anti-Mac-1) (33), NLDC145 (anti-DEC-205) (34), 3BD1 (35), and Moma-2 (36) were provided by the laboratories of origin and/or obtained from the American Type Culture Collection (Rockville, MD).

Immortalization of DC

Bone marrow cells flushed from the femurs and tibias of C57BL/6 mice (The Jackson Laboratories, Bar Harbor, ME) were depleted of RBC by ammonium chloride treatment. Nucleated cells (5×10^6) were then infected with an amphotropic, replication defective, retrovirus-expressing murine GM-CSF (CRIP MFG-murine GM-CSF) by cocultivation for 48 h in the presence of polybrene as previously described (37). Nonadherent cells were displaced by gentle pipetting and then placed into culture in 24-well dishes in the presence of RPMI, 10% FCS, and 10 ng/ml murine GM-CSF. Cells were refed every 2 days. Cultures developed an adherent monolayer and clusters of DC colonies. Cultures were dispersed when confluent and placed into medium lacking GM-CSF. After an additional 2 wk in culture in the absence of GM-CSF to expand the cell population, the floating cells were collected and infected with a retrovirus encoding *myc* and *raf* using supernatant (50%) from NIH J2 Leuk cells. After 36 h at 37°C, floating cells were collected, washed, and resuspended in DMEM high glucose supplemented with 10% FCS, L-glutamine, and antibiotics and passaged in tissue culture flasks. From these cell lines DC were subcloned by limiting dilution. For the subcloning and initial passage, conditioned media (50%) from the uncloned DC lines was added to the culture medium.

Immunofluorescence

Immunofluorescence staining was performed as previously described (38). Briefly, dendritic clones were incubated with mAb-containing supernatants for 45 min at 4°C, followed by FITC-conjugated goat anti-rat IgG or FITC-conjugated goat anti-mouse IgG (1/40; Cappel, Organon Teknika Corp., West Chester, PA). Samples were analyzed on a FACScan flow cytometer (Becton Dickinson).

RNA extraction and RT-PCR

Total RNA was extracted from cells and purified using the RNeasy Total RNA kit (Qiagen, Chatsworth, CA), and cDNA was prepared using reverse transcriptase. The oligonucleotide primers CCTTTGTGCCCAGCCT TATA (complementary to the positive strand of DEC-205 sequence, position 478) and CATTCTTTTCCCAGTTACCT (complementary to the negative strand of DEC-205 sequence, position 685) were synthesized by the Molecular Biology Core Facility of Dana-Farber Cancer Institute. A plasmid containing 5′ 2-kb DEC-205 cDNA (kindly provided by Drs. Wanping Jiang and Michel Nussenzweig, Rockefeller University, New York, NY) and cDNA from various clones were used as templates in PCR reaction. PCR-amplified products were analyzed on a 1.2% agarose gel.

Assays for phagocytosis

For ultrastructural analysis, DC2.4 cells were incubated with latex beads (3 μm in diameter) in 10-cm diameter culture dishes at 37°C for 30 min, and then washed and fixed with 1% paraformaldehyde. Subsequent embedding, ultrathin sectioning, and electron microscopy were performed at the Core Facility of Harvard Medical School (Boston, MA). For immunofluorescence analysis, cells were plated on coverslips, incubated with FITC-conjugated latex beads (3 μm in diameter) at 37°C for 30 min, and washed. Cells were examined on a fluorescence microscope (with the help of Dr. Joel Swanson, Harvard Medical School), and trypan blue (Fisher Scientific, Pittsburgh, PA; 25%, pH 5.0) was added to quench extracellular FITC-conjugated beads.

Preparation of Ag beads

Iron oxide beads (from PerSeptive Diagnostics, Cambridge, MA) were covalently conjugated to chicken egg OVA according to manufacturer's instruction. FITC (Sigma Chemical Co., St. Louis, MO) was passively absorbed to Latex beads (Polyscience, Inc., Warrington, PA; 1 μm in diameter) according to the manufacturer's instructions.

Ag presentation assays

APC were incubated in serum-free OptiMEM (Life Technologies, Gaithersburg, MD) supplemented with Nutridoma (Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C with or without the inhibitors brefeldin A (5 μg/ml, Sigma Chemical Co.), chloroquine (100 μM; Sigma Chemical Co.), cytochalasin B (5 μg/ml; Sigma Chemical Co.), LLnL (40 μM), LLM (40 μM), and MG132 (10 μM), followed by the addition of Ag. Ag was added in soluble form or bound to iron oxide beads. In some cases, OVA was loaded onto the cytosol by osmotic lysis of pinosomes (39), or SIIN FEKL was expressed in the cytosol using a vaccinia recombinant (a gift from Drs. Jon Yewdell and Jack Bennink) (40). After 5-h incubation at 37°C, the cells were washed, fixed with 1% paraformaldehyde solution, and added to microtiter plates. In some assays, live APCs (10^5/well) were incubated with varying concentrations of Ags in 200 μl of culture medium in flat-bottom microtiter plates. The culture medium was RPMI 1640 prepared as previously described and containing 0.25 μM indomethacin (41). Specific T-T hybrids were added to the microtiter plates and incubated for 20 h at 37°C, after which an aliquot (100 μl) of supernatant was collected and freeze-thawed. The IL-2 content in culture supernatants was assayed using an IL-2-dependent cell line CTLL as previously described (42, 43).

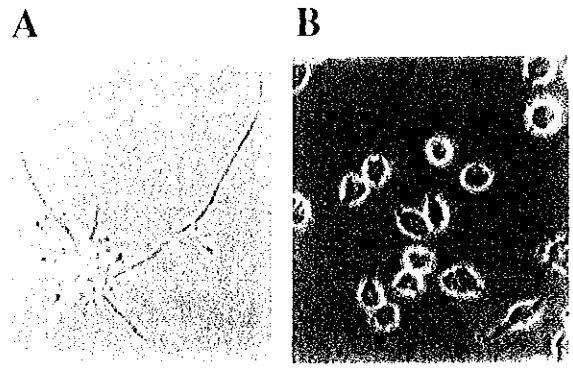

FIGURE 1. Photomicrographs of DC (*A*; DC2.4) and macrophage cells (*B*; A3.1A7). Magnification, ×680.

Results

Isolation of cell lines with DC morphology

It is well established that bone marrow-derived DC can be cultured in GM-CSF for short periods (44, 45). We attempted to develop long term cell lines by transducing bone marrow cells with GM-CSF. This resulted in the growth of cell populations that contained a subpopulation of cells with dendritic morphology. However, growth of these DC was not sustained beyond 6 wk, and we were unable to clone them.

We, therefore, modified this approach by superinfecting GM-CSF-transduced bone marrow cells with *myc* and *raf* oncogenes. The resulting cell populations contained cells with dendritic morphology that continued to grow in culture. By limiting dilution, 20 clones of DC were obtained in 4 to 5 wk. These cells had prominent dendritic processes and ruffled edges (Fig. 1*A*) that were not observed on macrophages (Fig. 1*B*); they attached to plastic and then detached over time, so that cultures contained both adherent and floating cells. Where examined, the DC clones do not continue to make measurable GM-CSF using a sensitive bioassay (data not shown). These DC clones have been in culture for >6 mo and can be frozen with cryopreservatives and thawed with good viability.

Characterization of cell surface molecules

We characterized the surface phenotype of these DC clones by immunofluorescence and flow cytometry. Individual fluorescence histograms for one clone are shown in Figure 2 (*A–K*), and the phenotypes of several different clones are summarized in Table I. All clones lacked T cell-specific (e.g., CD3) and B cell-specific (surface Ig) markers (data not shown). Most of these cells expressed high levels of MHC class I and class II molecules, the costimulatory molecules B7-1 and B7-2, as well as CD32 (FcγRII)

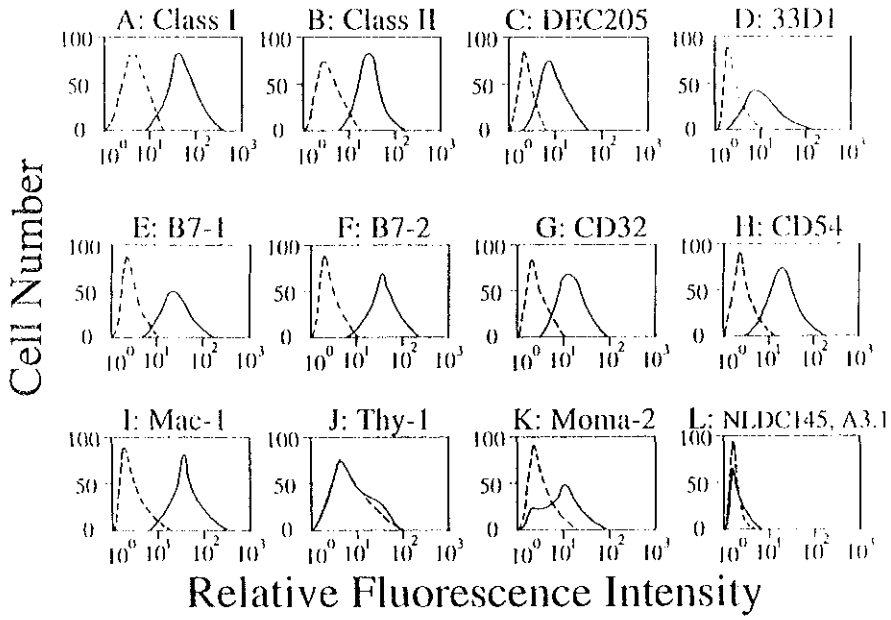

FIGURE 2. Immunofluorescence analysis of immortalized DC 2.4 cell line. DC 2.4 cells (*A–K*) and A3.1A7 macrophages (*L*) were stained by indirect immunofluorescence with mAb supernatants of the indicated specificities followed by appropriate FITC-conjugated secondary Abs as described in *Materials and Methods*. Dotted lines represent cell staining with FITC-conjugated secondary Abs only.

Table I. *Summary of surface phenotype of DC clones and A3.1A7*[a]

Marker	DC				
	DC1.2	DC2.4	DC2.5.1	DC4.1	A3.1A7
DEC205	+	+		+	
33D1	+/-	+		+	
B7-1	+ +	+	+	+	+/-
B7-2	+ +	+ +	+ +	+ +	+ +
Class I	+ +	+ +	+ +	+ +	+ +
Class II	+ +	+	+ +	+	+
CD32	+	+	+	+	+ +
Moma-2	+	+	+	+	+ +
Mac-1	+ +	+	+	+	+ + +
Thy-1					+/-
Ly 6A1		+			
CD54	+ +	+ +	+ +	+ +	ND

[a] Summary of surface phenotype of representative clones of DC compared with that of macrophage cells. A3.1A7. Four representative DC clones and A3.1A7 were stained by indirect immunofluorescence with mAb supernatants of the indicated specificities followed by appropriate FITC-conjugated secondary Abs as described in *Materials and Methods*. Relative fluorescence intensity is indicated with plus and minus signs; one plus represents approximately one log scale.

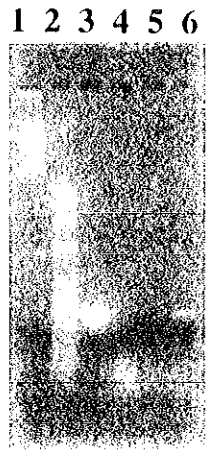

FIGURE 3. Analysis of RT-PCR products on 1.2% agarose gel. *Lane 1*, λ DNA *Hin*dIII digest; *lane 2*, PhiX174 RF DNA *Hae*III digest; *lane 3*, PCR product using plasmid containing DEC-205 cDNA as template; *lane 4*, RT-PCR product of mRNA from A3.1A7 macrophages; *lane 5*, RT-PCR product of mRNA from DC 2.5.1; *lane 6*, RT-PCR product of mRNA from DC 2.4.

and CD54 (intracellular adhesion molecule-1). These markers have all been reported on cultured DC (46–48), although they can also be expressed on other cell types.

The most specific markers for murine DC are the DEC-205 molecule recognized by the mAb NLDC145 (49) and the Ag detected by the mAb 33D1 (35). NLDC145 stained many of the DC clones, e.g., DC1.2, DC2.4, and DC4.1 (Fig. 2). The expression of DEC-205 in these cells was confirmed by RT-PCR (Fig. 3). The other dendritic marker, 33D1, also stained several clones. As expected, the macrophage cells A3.1A7 did not express DEC-205 (Fig. 2*L*) or 33D1 (data not shown). We concluded from these criteria that these cells represented cloned DC.

There were some lines with dendritic morphology that lacked the expression of both dendritic markers (Table I). For 33D1 this might simply reflect the sensitivity of the immunofluorescence assay because even on the DC where we detected expression, the intensity of staining was weak. However, these cells were also negative for the other dendritic marker, DEC-205, by both immunofluorescence and RT-PCR. The DEC-205-positive and -negative cells otherwise appeared to express the same surface molecules and were indistinguishable from one another on morphologic criteria. It is possible that the DEC-205-negative cells are of another lineage of cells (e.g., monocytes) or that there is heterogeneity in the expression of DEC-205 and 33D1 by primary isolated DC.

FIGURE 4. Ultrastructure of DC2.4 incubated with 3 μm-diameter latex beads for 30 min at 37°C and then washed and handled as described in *Materials and Methods*. Magnification, ×8000.

DC clones are phagocytic

To determine whether the DC clones were phagocytic, they were incubated with latex beads. When viewed by phase microscopy, the DC appeared to rapidly internalize these particles (data not shown). To determine whether the beads were ingested and not simply bound to the cell surface, we initially performed ultrastructural studies. Figure 4 shows an electron micrograph of DC2.4 cells incubated with latex beads at 37°C for 30 min. Numerous beads were visualized in most cells and appeared to be in vesicles. However, it is possible that surface-bound beads could give a similar appearance, depending on the plane of sectioning. Therefore, to further verify that beads were internalized into cells, we performed an immunofluorescence analysis. The DC were incubated with FITC-labeled latex beads and then analyzed by fluorescence microscopy. Trypan blue was added to quench the fluorescence of beads outside the cells. Figure 5 showed both phase (Fig. 5*A*) and fluorescence (Fig. 5*B*) images of representative cells. Most DC contained beads whose fluorescence was not quenched with trypan blue. The fluorescence of surface-bound or free beads was quenched. These analyses indicated that the DC clones are phagocytic.

Presentation of OVA on MHC class I and class II molecules

The availability of cloned DC allowed us to examine their Ag-presenting capabilities and particularly whether they could present exogenous Ags on MHC class I molecules. The presentation of Ag was determined by measuring the production of IL-2 from T-T hybrids specific for OVA peptides bound to MHC class I molecules. DC2.4 cells, a representative DC clone, presented on MHC class I molecules both soluble OVA and particulate OVA added to the culture medium (Fig. 6*A*). However, compared with soluble

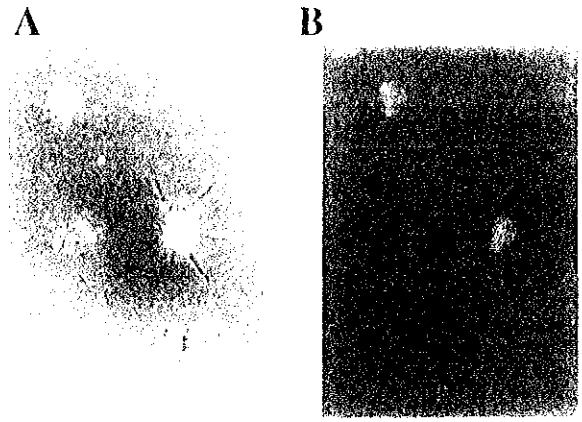

FIGURE 5. DC2.4 are phagocytic. Cells were incubated with 3-μm diameter latex beads conjugated with FITC for 30 min at 37°C and then quenched with trypan blue (25%, pH 5). Both phase (*A*) and fluorescence (*B*) pictures of the same field were taken. Many intracellular fluorescent beads are of the plane of focus. Extracellular beads were not fluorescent in any plane of focus. Magnification, ×630.

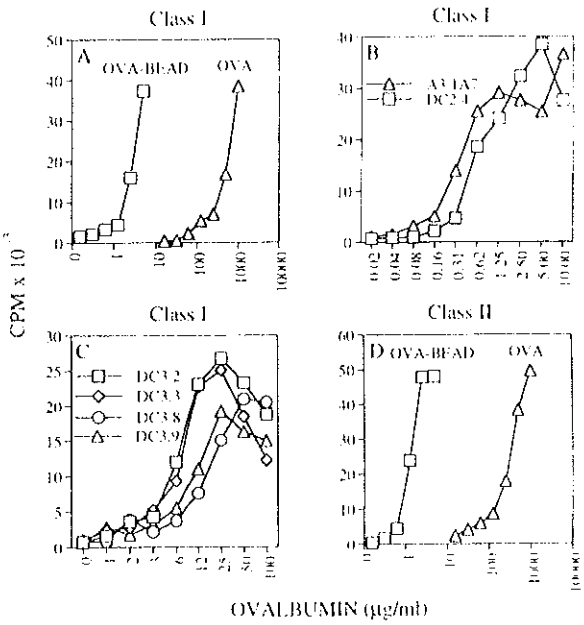

FIGURE 6. Presentation of exogenous OVA and soluble OVA on MHC class I and class II molecules. APCs (5×10^4/well) and RF33.70 (*A*, *B*, and *C*; 5×10^3/well) or MF2.2D9 (*D*; 5×10^4/well) and the indicated amount of bead-conjugated OVA or soluble OVA were cultured in microtiter plates (200 μl). Cultures were then handled as described in *Materials and Methods*. *A*, Presentation of bead-conjugated OVA (open square) and soluble OVA (open triangle) by DC2.4 cells on MHC class I molecules; *B*, presentation of bead-conjugated OVA by DC2.4 (open square) and A3.1A7 (open triangle) on MHC class I molecules. *C*, Presentation of bead-conjugated OVA by various DC clones on MHC class I molecules. *D*, Presentation of bead-conjugated OVA (open square) and soluble OVA (open triangle) by DC2.4 cells on MHC class II molecules.

OVA, the presentation of bead-bound OVA was 100- to 1000-fold more efficient (Fig. 6*A*). Other DC clones were similarly capable of presenting OVA on MHC class I molecules (Fig. 6*C*). This

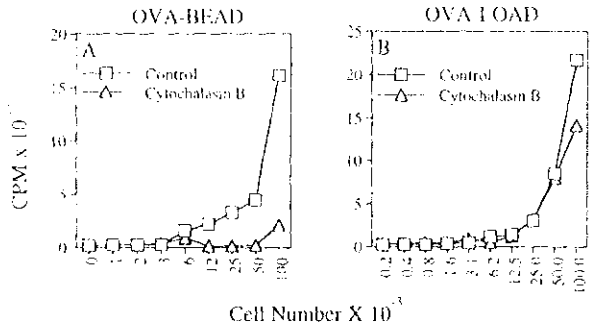

FIGURE 7. Effect of cytochalasin B on exogenous OVA presentation on MHC class I molecules. DC2.4 cells (2×10^6/ml) were pretreated with 5 μg/ml cytochalasin B for 30 min (open triangle) or medium alone (open square) followed by the addition of bead-conjugated OVA (50 μg/ml; *A*) or hypotonic loaded OVA by lysis of pinosome (2 mg/ml; *B*), further incubated for 6 h, and then fixed with 1% paraformaldehyde. Cells were then titrated in microtiter plates (100 μl), and the T-T hybrid cells (5×10^4/well), RF33.70 (*A* and *B*), were added to the cultures (200 μl total) and handled as described in *Materials and Methods*.

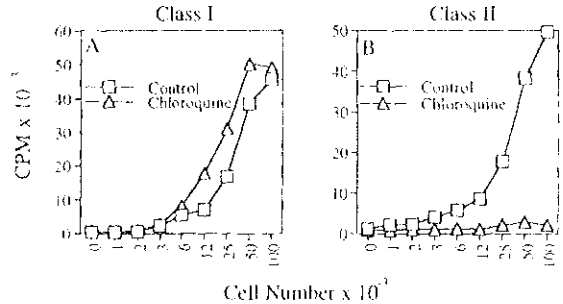

FIGURE 8. Effect of chloroquine on exogenous OVA presentation on MHC class I and class II molecules. DC2.4 cells (2×10^6/ml) were pretreated with 100 μM chloroquine for 30 min (open triangle) or with medium alone (open square) followed by the addition of bead-conjugated OVA (50 μg/ml), further incubated for 6 h, and fixed with 1% paraformaldehyde. Cells were then titrated in microtiter plates (100 μl), and the T-T hybrids (5×10^4/well), RF33.70 (*A*) and MI.2.2D9 (*B*), were added to the cultures (200 μl total) and handled as described in *Materials and Methods*.

Ag-presenting capability was similar to that of A3.1A7, a macrophage cell line (Fig. 6*B*) (14). Since the DC were pure clones, we conclude that the pathway for presenting exogenous Ag on class I molecules is active in these APCs.

We also examined the ability of the DC to present Ag on MHC class II molecules. These cells presented soluble OVA to an OVA plus IA^b-specific T-T hybrid (Fig. 6*D*). Similar to the class I pathway, DC2.4 presented bead-bound OVA more efficiently than soluble OVA. Therefore, a DC can acquire exogenous Ag and present it simultaneously on both class I and class II molecules. Compared with similarly derived macrophage cell lines, the DC expressed higher levels of MHC class II molecules and were more potent at class II Ag presentation (data not shown). Without IFN-γ stimulation, the macrophage cell lines were either incapable or only weakly able to present Ag on class II (14).

Presentation of particulate OVA was inhibited by cytochalasin B

Phagocytosis was previously shown to be crucial for the presentation of particulate OVA by macrophages (11, 14). Here we examined the effect of cytochalasin B, an inhibitor of phagocytosis, on the presentation of OVA by DC. Treatment with cytochalasin B (5 μg/ml) inhibited the presentation of bead-bound OVA on MHC class I molecules (Fig. 7*A*). In contrast, this agent did not affect presentation when OVA was loaded directly into the cytosol (Fig. 7*B*), which indicates that it is interfering with an early event in the exogenous pathway and not at other steps in the class I pathway.

Presentation by DC of exogenous Ag on MHC class I molecules is chloroquine insensitive

The presentation of exogenous Ags on MHC class I molecules in some cases involves proteolysis of the Ag in the cytosol (8–10), while in other cases the proteolysis appears to occur in the endocytic compartment (7, 11–13). It was of interest to determine which of these mechanisms was operative in DC. To investigate whether presentation required proteolysis in acidic vesicles, we treated the DC with chloroquine during exposure to exogenous Ag. Chloroquine raises the pH in the endosomal and lysosomal compartments and thereby inhibits protein hydrolysis by cathepsins, which require an acidic environment for activity (50, 51). Treatment with chloroquine did not inhibit the presentation of particulate OVA on MHC class I molecules (Fig. 8*A*). In fact, this agent actually slightly enhanced this presentation, suggesting that proteolysis in vesicles might be limiting the availability of Ag for class I presentation. In contrast, chloroquine treatment completely inhibited the presentation of bead-bound OVA on class II molecules, as expected (Fig. 8*B*) (52). This latter finding serves as a positive control for the effectiveness of chloroquine in blocking proteolysis in vesicles. These results suggest that the class I-presented peptides are being generated outside of the endocytic compartments in DC.

Role of the proteosome in class I presentation by DC

The other major pathway for degrading proteins in cells is mediated by proteasome in the cytosol and nucleus (53). This pathway is responsible for generating the majority of class I-presented peptides from endogenous cellular and viral proteins (2) and has been implicated in the presentation of exogenous Ags by macrophages (9). Therefore, we next examined the effects of peptide aldehyde inhibitors of proteasome (2) on the presentation of exogenous OVA by DC. As shown in Figure 9*A*, two of these inhibitors, LLnL and MG132, inhibited the presentation of particulate OVA on MHC class I molecules. In contrast, a closely related peptide aldehyde, LLM, did not inhibit the presentation. This agent serves as a specificity control because it has activity similar to those of LLnL and MG132 on thiol proteases, but is much less potent against the proteasome (2). Furthermore, LLnL and MG132 did not inhibit the presentation of the OVA peptide SIINFEKL expressed in cytosol from a minigene in a vaccinia virus construct (40) (Fig. 9*B*). These results indicate that LLnL and MG132 inhibit the presentation of exogenous OVA by inhibiting peptide generation by the proteasome and not by affecting other steps in the class I pathway.

Brefeldin A inhibited bead-conjugated OVA on both MHC class I and class II molecules

Brefeldin A blocks the exocytosis of proteins from the endoplasmic reticulum and Golgi complex and thereby prevents newly assembled peptide-MHC molecules from reaching the cell surface (54). The presentation of exogenous Ag on MHC class I molecules by the vacuolar pathway has been reported to be insensitive to this inhibitor (7, 11, 13), while presentation by the cytosolic pathway is inhibited by brefeldin A (8–10). We therefore examined the

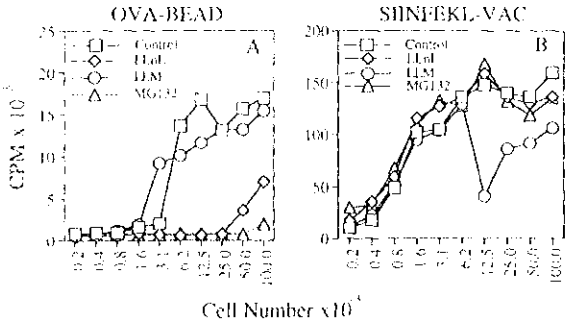

FIGURE 9. Exogenous OVA presentation was inhibited by proteasome inhibitors. DC2.4 cells were preincubated with medium alone (open square), 40 μM LLnL (open diamond), 40 μM LLM (open circle), or 10 μM MG132 (open triangle) for 30 min followed by the addition of bead-conjugated OVA (50 μg/ml) for 6 h (*A*) or infection with recombinant vaccinia virus encoding the SIINFEKL peptide (10 plaque-forming units/cell) for 3 h (*B*). Cells were then fixed with 1% paraformaldehyde and incubated with RF33.70 (10^5/well) for 20 h (200 μl). Cultures were then handled as described in *Materials and Methods*.

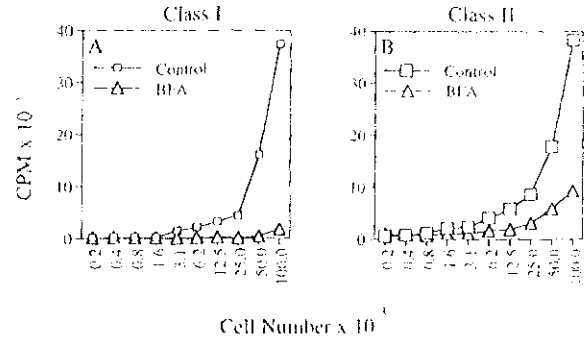

FIGURE 10. Effect of brefeldin A on exogenous OVA presentation on MHC class I and class II molecules. DC2.4 cells (2×10^6/ml) were pretreated with 5 μg/ml brefeldin A for 30 min (open triangle) or medium alone (open square) followed by addition of beads conjugated OVA (50 μg/ml), further incubated for 6 h, and fixed with 1% paraformaldehyde. Cells were then titrated in microtiter plates (100 μl), and the T-T hybrids (5×10^4/well) RF33.70 (*A*) and MF2.2D9 (*B*) were added to the cultures (200 μl total) and handled as described in *Materials and Methods*.

effect of this agent on the presentation of exogenous OVA by DC. Brefeldin A (5 μg/ml) completely inhibited the presentation by DC2.4 cells of particulate OVA on MHC class I molecules (Fig. 10*A*) and also inhibited the presentation of OVA on MHC class II molecules (Fig. 10*B*), suggesting a role for newly synthesized class II molecules in presentation of Ag by these DC.

Discussion

Properties of cloned DC

DC are present in both lymphoid and somatic tissues, but in relatively low abundance (55). Consequently, it is difficult to obtain large numbers of these cells with a high degree of purity. Many types of studies would be facilitated by the availability of reliable methods to isolate large numbers of homogeneous DC. Larger numbers of these cells can be obtained by culturing precursors from peripheral blood or bone marrow in GM-CSF and other cytokines (44, 45). This has been an important advance; however, these cultures typically contain other contaminating cell types, and this approach has not allowed the isolation of cloned DC lines. We also failed to grow clones of DC from bone marrow cultures transduced with GM-CSF. Presumably, GM-CSF stimulation is not sufficient for immortal growth of DC or their progenitors. This is consistent with previous studies examining the reconstitution of lethally irradiated mice with bone marrow that had been infected with a replication defective, retrovirus-expressing GM-CSF (56). Although a myeloproliferative disease occurred secondary to constitutive GM-CSF secretion in the primary recipients, this did not evolve into a clonal leukemia. Moreover, the myeloproliferative disorder could not be transplanted into secondary recipients (56). However, we found that supertransfection of *myc* and *raf* into GM-CSF-transduced cultures immortalizes these cells. In this case GM-CSF probably acts in a paracrine manner to expand infectable DC because immortal growth is maintained without the clones producing detectable levels of this cytokine. Similar approaches to immortalize DC using oncogenes (without GM-CSF) have been reported by others (57, 58), although whether the isolated cells are bona fide DC has not been firmly established in all cases.

The initial criteria we employed for identifying DC was morphologic. These clones had very obvious dendritic processes and veils, which was an appearance we had never observed in cells growing from fresh bone marrow infected with the same *myc*- and *raf*-expressing retrovirus (a procedure yielding macrophage and B cell clones) (14) (our unpublished observations). That at least some of these cells were indeed DC was confirmed by their expression of specific DC markers, DEC-205 and 33D1. These cells also expressed other molecules that are typical for DC, including high levels of B7 family members and MHC class I and class II molecules. They also expressed other receptors that are not present on freshly isolated DC, such as CD32 and Mac1, but which are found on cultured DC (47, 57, 59, 60).

The DC clones can avidly internalize micrometer-sized particles. This process requires microfiliments, and the particles are found in cells in large vacuoles. Therefore, the DC are phagocytic. This property has previously been observed for cultured DC and DC resident in tissues (61, 62). We show that this activity is important for the presentation on MHC molecules of peptides from particulate Ags (further discussed below).

We also isolated clones with similar morphology but that lacked expression of DEC-205 and 33D1. Given the similarity of their appearance and their expression of other cell surface molecules to the DEC-205-positive clones, we favor the possibility that these clones represent different subsets or different stages of maturation of DC. This interpretation would be consistent with other data demonstrating phenotypic heterogeneity in freshly isolated and cultured DC (63–65). However, in the absence of other objective criteria, it is difficult to rule out the possibility that these DEC-205-negative cells represent some other unrelated cell lineage or that their phenotype is abberent and an artifact of the immortalization conditions.

The immortalized DC lines are homogeneous and easily grown. These properties should be useful to studies exploring the cell biology and biochemistry of these cells. We have used them to analyze the Ag-presenting pathways that are operative in these cells.

Presentation of exogenous Ags by DC

The major question addressed by the present study is whether DC are capable of presenting Ags from the extracellular fluids on MHC class I molecules. Previously, cells with this capability were detected in fractions from both spleen and thymus enriched in DC

(5, 16). However, these same fractions contained some macrophages (5), and macrophages have been shown to have this Ag-presenting activity (11, 14). Our present results conclusively demonstrate that cloned DC that are free of macrophage contamination can present exogenous Ags on class I molecules. This pathway of presentation was detected in multiple different clones.

Soluble OVA added to culture medium was presented on class I molecules by DC, but required high concentrations of Ag. Approximately 1000-fold less Ag was required when the OVA was conjugated to a microsized particle that was internalized by phagocytosis. This enhanced presentation was blocked by cytochalasin B, which disrupts phagocytosis but not other endocytic processes. These results are similar to earlier findings with macrophages (14) and indicate that class I molecules can monitor the contents of phagosomes and potentially other endocytic compartments in DC. One such compartment might be the macropinosome, because this is a site in other cells that has been implicated in the delivery of exogenous proteins into the class I pathway (8), and macropinocytosis occurs in DC (66).

Two distinct pathways have been described for the presentation of internalized Ags on class I molecules of macrophages. One is independent of the proteasome and TAP and is resistant to brefeldin A (7, 11–13). In this case it is thought that the presented peptides are generated in the endocytic compartment and then bind to class I molecules on the plasma membrane. The other pathway requires proteasome and TAP and is sensitive to brefeldin A (8 - 10). In this case, Ags are transferred from phagosomes into the cytosol where they follow a common final pathway with endogenous Ags. The presentation of OVA particles by DC is sensitive to inhibitors of the proteasome and brefeldin A, but resistant to chloroquine. Therefore, it appears that in these APCs OVA is following the phagosome to cytosol pathway. Whether DC also have a vacuolar pathway for class I presentation remains to be determined.

Compared with macrophage lines, the cloned DC present exogenous Ag with similar efficiency to MHC class I-restricted T-T hybridomas. Nevertheless, it is likely that these cells will be more effective in stimulating responses to these Ags because of their potent immunostimulatory properties. T-T hybrids only require stimulation through their TCR, while normal T cells require additional signals, such as B7-1 and B7-2, which are expressed at high levels on DC. Moreover, the DC clones are constitutively better class II-presenting cells than macrophages, and this correlates with higher levels of expression of MHC class II molecules.

There is considerable evidence that the pathway for presenting exogenous Ags on class I molecules is active in vivo (6). Macrophages and DC isolated from immunized animals present the injected Ag on their class I molecules. Moreover, injection of antigenic particles primes CTL responses (6, 20). This pathway probably plays an important role in generating immunity in several pathologic situations, including responses against tissue transplants, tumors, and possibly even viruses (3, 17–19). In some of these situations, the exogenous pathway may be the major mechanism for initiating responses. This may be because the somatic cells that are producing the Ag are themselves poor stimulators of immunity, and professional APC, such as the DC, are needed to prime responses (15).

The exogenous class I pathway can potentially be exploited to generate CTL immunity to proteins in vaccines. Conventional vaccine preparations generally fail to elicit CTL responses, presumably because the Ags fail to be presented in vivo. However, particles can be used to target proteins into phagocytes, and this is effective in conferring protective CD8 T cell immunity (6, 20). The existence of the phagosome to cytosol pathway in DC makes this approach particularly attractive. Studying this pathway in the cloned DC should help to optimize approaches for targeting Ags into these key APCs.

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MATERIAL TRANSFER AGREEMENT

Dana-Farber Cancer Institute, Inc.

The University of Western Ontario at 1151 Richmond Street, London ON N6A 3K7 and its investigator(s) Dr Lakshman Gunaratnam (hereinafter collectively referred to as "Recipient"), in consideration of the receipt of biological materials (which material has been provided to Dr. Mansour Haeyrfar of The University of Western Ontario by way of Material Transfer Agreement dated September 29, 2006 and Dana-Farber Cancer Insitite (hereinafter "DFCI") herby consents to Dr. Haeyrfar providing said Material to Dr. Lakshman Gunaratnam) hereby agree to the following terms and conditions:

1. The biological materials to be provided to Recipient are: DC2.4. Material(s) shall mean the above referenced biological materials plus progeny, unmodified derivatives and any accompanying know-how or data.

2. The Materials shall be used exclusively for non-commercial research by Recipient to study Testing conditions that lead to DC maturation after renal transplantation.. The Material(s) shall be used solely by the named investigator and those under his or her direct supervision. Materials will not be used for *in vivo* testing in human subjects. Use will be in compliance with all applicable Federal, State and local laws and regulations, including, but not limited to animal welfare laws and regulations.

3. The Materials are the property of DFCI. Ownership of modifications and derivatives of Materials will be determined by the parties hereto depending upon (a) their relative contribution to the creation of said modifications and derivatives, which is to be considered but not required in said negotiation; and (b) any applicable laws and regulations relating to inventorship.

4. Recipient shall not sell or otherwise distribute Materials to a third party for any purpose. This Agreement and the resulting transfer of Material constitute a non-exclusive license to use the Material solely for the basic research or other not-for-profit purposes described herein. Recipient shall not use Materials for any products or processes for profit-making or commercial purposes.

5. This agreement is not assignable.

6. DFCI has, or may, make Materials available to others, both profit and non-profit.

7. To the extent supplies are available, DFCI agrees to make the Material available, under a separate agreement, to other scientists for teaching or not-for-profit research purposes only. Recipient will acknowledge DFCI as the source of the Material in all publications containing any data or information about the material unless DFCI indicates otherwise.

8. Recipient will arrange the return to DFCI or disposal of all unused Material whenever investigation for which it has been supplied discontinues or is terminated. In the event investigator(s) transfer to another institution, a new Material Transfer Agreement is to be executed.

9. The Material hereunder provided is experimental in nature, and it is provided WITHOUT ANY WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. DFCI MAKES NO REPRESENTATION AND PROVIDES NO WARRANTY THAT THE USE OF THE MATERIAL

WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHT.

10. To the extent allowable under applicable laws, Recipient agrees to indemnify, defend, and hold harmless DFCI and its trustees, officers, staff, representatives and agents against all damages, expenses (including without limitation legal expenses), claims, demands, suits or other actions arising from Recipient's acceptance, use and disposal of the Materials and their progeny or derivatives, except insofar as such claims result directly from the gross negligence or willful misconduct of DFCI.

Accepted by: Institution: The University of Western Ontario

Authorized

Institutional Officer: _____ Investigator: Dr Lakshman Gunaratnam

Title: _____ Title: _____

Signature: _____ Signature: _____

Date: _____ Date: _____

Acknowledgement: Dr. Mansour Haeyrfar, in consideration of section 1 as provided, hereby acknowledges and consents to the transfer of DC2.4 cells to Dr. Lakshman Gunaratnam.

By:_____

Approved by: DANA-FARBER CANCER INSTITUTE, INC.

_____ Date: _____

Anthony A. del Campo, MBA

Vice President, Office of Research and Technology Ventures

Outbound MTA Agr [Agr ID], 18/05/2010

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
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 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	LAKSHMAN GUNARATNAM
SIGNATURE	
DEPARTMENT	MEDICINE
ADDRESS	1400 WESTERN RD
PHONE NUMBER	519-661-2111, EXT-89120
EMERGENCY PHONE NUMBER(S)	519-636-4274, 226-663-1374
EMAIL	LGUNARAT@UWO.CA

Location of experimental work to be carried out: Building(s) SDRI Room(s) 230, 229, 231

*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: KRESCENT / KFOC SUBMITTED, START-UP
GRANT TITLE(S): Mechanism of inhibition of danger signaling and renal transplant rejection by Kidney Injury Molecule-1

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Xizhong Xhang, MD, PhD
Ola Ismail, B.Sc.

*** 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
(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)*	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	O Yes ⊘ No	O Yes ⊘ No	O Yes ⊘ No	2 L	Invitrogen Commercial	⊘ 1 O 2 O 3
Lentivirus	⊘ Yes O No	⊘ Yes O No	O Yes ⊘ No	10^6 - Ifv	Commercial (SCAST)	O 1 ⊘ 2 O 3 +
	O Yes O No	O Yes O No	O Yes O No	10		O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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	O Yes ⊘ No		Not applicable
Rodent	⊘ Yes O No	Mouse kidney, spleen, blood, bone marrow	2010-037 Pending
Non-human primate	O Yes ⊘ No		
Other (specify)	O Yes ⊘ No		

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

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 O No	786-O, 769-P, HEK293 (HKL)	ATCC
Rodent	⊘ Yes O No	293T, in IMCD-3, JAWS II CMT-93, RAW, WT-9.	ATCC
Non-human primate	O Yes O No		
Other (specify) Porcine	⊘ Yes O No	LLC-PK1,	ATCC

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

* Please see attached sheet. for details

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
(DH5 α) E. Coli	pCDNA3	Commercial	KIM-1	Unknown oncogenic Induces phagocytosis of apoptotic cells

* 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? O YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Lentivirus	pLVX-Puro Proprietary	Commercial Invitrogen/Clontech Santa Cruz Biotech	Kim-1 or shRNA/siRNA	See previous Section 4.2

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

JQ

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? O YES ✓ NO II+

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? ✓ YES ~~✓ NO~~ If no, please proceed to section 7.0

6.2 Name of animal species to be used Musculus

6.3 AUS protocol # 2010-037 - Pending

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:
N/A

*** 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 of the following animals or their organs, tissues, lavages or other body fluids including blood be 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~~ O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) C3 Exotoxin, : Phalloidin
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 C3 50ng (mice) / Phalloidin 2.5mg/kg (mice)

8.4 How much of the toxin is handled at one time*? C3 10-20ng / Phalloidin (7 μmoles)

8.5 How much of the toxin is stored*? C3 25 μg / Phalloidin (500 mL x 14 μM)

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? 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 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? 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 NO, please forward the permit to he Biosafety Officer when available.

10.9 Please 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_____
If no, please proceed to Section 12.0 ✓ NO

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  _____

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

13.0 Containment Levels

11.1 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+ O 3
SDRI 231A

13.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus ~~ee~~ inspected June 29, 2010 dl
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: 04/19/2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.
-

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

First Aid → Cleanse wound → Emergency room visit → Report to occupational health

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidder
Date: 29 June 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: J Stanley
Date: June 29, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: BIO-UWO-0256 Expiry Date (3 years from Approval): June 28 2013

Special Conditions of Approval:

Follow SOP attached. - Protocol... Lentiviruses.

Adhere to maximum amounts to handle / store toxins (per attached e-mail).

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

Subject: Re: Biohazardous Agents Registry Form: Gunaratnam
From: Lakshman Gunaratnam <Lakshman.Gunaratnam@lhsc.on.ca>
Date: Thu, 03 Jun 2010 14:46:33 -0400
To: jstanle2@uwo.ca

* new info June 3, 2010

Sorry
O.1ug (70nM x 1/1000L x 1490 MW) max per use.
lakshman

Lakshman Gunaratnam, MD.,M.Sc.
Assistant Professor of Medicine
Nephrologist, University Hospital
London Health Sciences Centre
Schulich School of Medicine and Dentistry
University of Western Ontario
Siebens Drake Research Institute
1400 Western Road, Room 230B,
London, ON N6G 2V4

Phone: (519) 661-2111, Ext.89120
Email: gunaratl@lhsc.on.ca

> Jennifer Stanley <jstanle2@uwo.ca> 06/03/10 1:52 PM >>>

Thanks Dr. Gunaratham

Can you tell me the maximum amount of phalloidin handled at one time - please provide the amount in ug or mg

I think you missed this question?

Jennifer

On 6/3/2010 1:48 PM, Lakshman Gunaratnam wrote:

> Please see below for confirmation:
>
> 1. Please confirm the following for the exotoxin:
> LD50 - 50 ng/kg (for mice)
> max amount handled at one time - 50-100 ng
> max amount stored - 20 ug (0.1mg/ml x 0.2ml)
>
> 2. Please let me know the following for the phalloidin:
> LD50 - 2 mg/kg (for mice)
> max amount handled at one time - please provide the amount in ug or mg
> max amount stored - please provide the amount in ug or mg = 10ug (14uM stock x 1490 MW x 0.5ml)
>
> Thank you.
>
> Lakshman Gunaratnam, MD.,M.Sc.
> Assistant Professor of Medicine
> Nephrologist, University Hospital
> London Health Sciences Centre
> Schulich School of Medicine and Dentistry
> University of Western Ontario
> Siebens Drake Research Institute
> 1400 Western Road, Room 230B,
> London, ON N6G 2V4

0.1ug (see above)

new info
June 10/10

- DC.4 cells
- They are transformed (SV40 T antigen) mouse dedritic cells. They were made by Dr. Ken Rock while at Dana Farber. Sorry for the oversight.

1. Please confirm the cell lines that you use (it was difficult to decipher the cell lines in Table 2.3)

- MDCK
- LLC-PK1
- RAW
- HEK293, HEK293T, 293T
- Jurkat
- JAWSII
- HK-2
- WT-9 DC4
- mIMCD-3
- CMT-93
- 769-P and 786-O renal cell cancer ell lines
- We will use/establish stably transfected cells expressing KIM-1 or KIM-1-GFP

Mouse Primary Cells

- Renal tubular epithelial cells from kidney
- Dendritic cells from bone marrow, lymph nodes or spleen
- CD4 and CD8 T cells from lymph nodes or spleen or kidney
- Splenocytes
- Thymocytes

2. Please send some information on the lentiviral vectors, etc. - such as an MSDS and/or website information (see attaches printouts)
- see attached printouts

3. Please send a description of the work you do that describes how the biohazards are used, stored and disposed of. Be sure to describe the modifications you do with the lentiviral vectors.

DMSO: Will be used as a preservative to cryopreserve cells. DMSO will be used at 10% in fetal bovine serum. DMSO accumulates in the medium at <1% concentration and will be discarded in the sink with refuse media after addition of bleach. DMSO will be stored in the stored in the contained provided by the manufacturer and stored as described in MSDS (provided).

C3 Exotoxin: C3 is used to block RhoA activity in cells in culture at 1microgram/mL. Media containing C3 will be discarded as stated above (DMSO) given that C3 at these diluted concentrations is not harmful to the environment. C3 is stored at -20 degrees Celsius as indicated by MSDS.

E.coli: Will be used for cloning and plasmid preparation. Standard precautions will be used under biohazard safety level 1.

Lentivirus: Lentivirus technology will be used to introduce or silence (SiRNA/shRNA) our gene of interest, KIM-1, into established cell lines or primary cells in culture. We are planning on purchasing Lentivirus for SiRNA/shRNA from Santa-Cruz Biotechnology (http://datasheets.scbt.com/sc-61691-sh.pdf). Expression vectors will be purchased from Clonetech (http://tools.invitrogen.com/content/sfs/manuals/virapower_lentiviral_system_man.pdf).

As far as we know KIM-1 is not a proto-oncogene. We will follow all standard safety practices when handling or discarding lentiviral particles (described in attached document from Queen's University Environmental Health and Safety).

Questions raised in review of protocol:
1. Where will it be done?
All lentiviral work will be done in a designated bio-safety cabinet in a closed room. All personnel will wear N95 masks at all times (during interaction) and use double-glove technique. All contaminated equipment will be placed in bleach solution as described in
2. Which cells (specifically) do you intend to infect with lentiviral particles?
Lentiviral technology will be used to express KIM-1 or SiRNA/shRNA targeting KIM-1 in mouse primary cells and cell lines that are not easily transfectable at high efficiency. The cell types to be infected with lentivirus are: Primary mouse kidney tubular epithelial cells, mouse primary T cells, HEK-293 (ATCC), LLC-PK1 (ATCC), 769-P(ATCC), 786-O (ATCC), CMT-93 (ATCC).

Describe your overall experimental design - a grant summary may suffice. Please see attached summary from recently submitted grant/award. The primary purpose of the lentiviral expression system is to decipher how the presence or absence of KIM-1 in our cells affects phagocytosis of apoptotic/necrotic cells and how this affects downstream signaling events that can lead to inflammation at the organism level (i.e. in transplantation). We do not plan to use lentivirus in animals.

We will need and adhere to biohazard containment level 2 precautions in conducting all this work (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm) and http://www.clontech.com/products/detail.asp?product_id=172594&tabno=2 (Clonetech).

Prepared by
Lakshman Gunaratnam, MD

Western

Protocol for Handling Recombinant Replication-deficient Lentiviruses

Lentiviral vectors are different from the commonly used adenovirus-based gene delivery systems because the gene of interest becomes stably integrated into the host cell's genome. The efficiency of lentiviral systems is impart due to the fact that they are actively imported into the nuclei of dividing, as well as non-dividing cells, as opposed to traditional retroviruses.

The lentiviral genome contains nine genes but only three of those are required to package a replication-deficient virus. The three essential genes are Gag, Pol and Env and they can all be provided in trans. Gag encodes a capsid proteins and Pol is required for the viralreverse transcriptase, RNase, protease and integrating functions. The Env, or, envelope gene encodes a transmembrane glycoprotein that also determines the tropism of the viral particle (ie. the specificity of the virus for a particular host cell). **In the ViraSafe Ecotropic Packaging system from Cell Biolabs, Inc. the *env* gene encodes a glycoprotein from murine ecotropic retrovirus, thus providing a viral particle that can transduce only mouse and rat cells with high efficiency**. However, other types of retroviral envelope genes can be used. If the *env* gene is from a xenotropic retrovirus the lentivirus vector will only infect non-mouse cells. If *env* gene is from an amphotropic retrovirus then it will infect cells in a species independent fashion. More frequently though the envelope gene G from Vesicular Stomatitis Virus (VSV) is used to pseudotype the lentivirus core with an envelope protein that provides a broader host range with better infectivity in some cases. The actual lentiviral genome that gets packaged is devoid of any coding sequence and the U3 region of the genomic RNA is deleted so that when the RNA genome is reverse transcribed it generates a self-inactivating DNA version of the RNA genome that after integration cannot lead to the generation of viral progeny. The remainder of the viral genome (*ie.* cis-elements only) is used to construct and direct the packaging of different lentiviral cloning vectors when the cloning vectors along with plasmids that provide the gag, pol and env genes in trans are co-transfected into packaging cell lines (usually HEK 293T cells). Cells containing and expressing all the necessary components then produce new infectious replication-defective viral progeny over the next 24 to 72 hrs.

Note! Only laboratory personnel that have been informed about safety precautions and working routines, and have permission from the person in charge are allowed to enter the laboratory during Lentiviral work production. <u>This also includes cleaners and service-personnel</u>.

Principle:

All procedures for handling or manipulating Lentivirus should be carried out at Biosafety Level 2 (BL2) with the use of Containment Level 3 operational practices. All work will be done in a biological safety cabinet (BSC) by authorized personnel wearing gloves, safety glasses, shoe covers, and overgowns that cover the front and close at the back. Personal items (eg. purses) will not be brought into the containment room. All protective clothing will be removed upon completion of the work and left in the room or disposed of as waste (shoe covers, gloves). Protective items to be re-used will be autoclaved. Overgowns will be kept on a coat rack within the containment room. No work with these viral vectors is permitted on the open bench. The door to the laboratory must remain closed

Working precautions for handling Lentivirus:

1. All experimental materials shall be handled with care.
2. The door to the containment room shall remain locked.
3. Within the BSC:
   a. For small quantities of low (cell lysate) and high (purified) titer Lentivirus, use sterile, aerosol barrier-containing pipette tips.
   b. For larger amounts (more than 1ml) of low titer lysates use sterile serological disposable pipettes.
   c. The maximum amount of infected growth media handled at one time should never exceed 500 mL.
4. Using a dunk tank, plastics will first be either filled (eg. pipette tips and serological pipettes) or rinsed (eg. plates and flasks) with Wescodyne Solution (20% Wescodyne/40% ethanol/40% water), drained, and then put into a high-density 4mil polyethylene plastic biohazard bag lined with a cardboard box prior to autoclaving.
5. Concentration of the viral particles will be done using either appropriate ultracentrifuge rotor or by using Amicon Ultra-15ml 100k MWCO centrifugal filter devices. All centrifugation shall be done in closed buckets with aerosol-tight lids. Loading and unloading of samples into the sealed buckets will be done in the BSC. The sealed centrifuge buckets will be sprayed with 70% ethanol before removing from BSC.
6. Sharps shall be eliminated from experimental procedures to prevent injuries. No needles or Pasteur pipettes will be used in the production and use of lentivirus.
7. Double gloves shall be worn at all times when working with viral vectors. Gloves will be sprayed with 70% ethanol and then the outer glove removed inside the hood by using the inside-out technique before disposing into biohazard waste dunk tank located inside the BSC. Remove lab coat and boots and dispose of in biohazard waste. Spray inside glove with 70% ethanol and remove using the inside-out technique, dispose into biohazard waste bag. Wash hands immediately after removing gloves and before leaving work area. Never wear gloves outside of the laboratory, or touch things with gloved hands.

8. During any lentiviral work, signs and labels shall be placed to indicate each area where viral vectors are used and stored (BSC, incubators, freezer, laboratory entrance doors, etc.)

Decontamination and disposal procedures:

All materials that come in contact with viral particles must be properly decontaminated prior to disposal. This requires that all material must be autoclaved prior to leaving the level 2 plus 3 room where the work is being done. This requires that the level 2plus 3 room must have an autoclave in it. Alternatively a portable autoclave may be used.

1. ***Disposal/decontamination of solid waste such as, paper tissues, pipette tips, etc.***: All solid waste (including disposable plastic wares) should be discarded in biohazard bags for the appropriate treatment (autoclaving) according to PHAC/CFIA guidelines, institutional practices and guidelines prior to disposal. Information on hazardous waste disposal is found in the Hazardous Materials Management Handbook:
   http://www.uwo.ca/humanresources/docandform/docs/ohs1/manuals/hazardous_handbook.pdf
   Personnel must take the Biosafety training courses from Occupational Health and Safety and General Laboratory Safety and Hazardous Waste Management.
2. ***Disposal/decontamination of liquid waste***: All liquid materials (Lentivirus-containing media, buffers, washes) should be decontaminated inside safety cabinet by addition of wescodyne or Quatricide PV Solution prior to autoclaving.
3. ***Work surfaces inside cabinets*** should be decontaminated with Quatricide PV Solution allowing for a wet contact time of at least ?? minutes to ensure virus inactivation, followed by 70% ethanol again with at least ?? minutes of contact time.
4. ***Instruments, equipment*** and any other items that are not disposable and contact Lentivirus will be decontaminated with Quatricide PV Solution (again observe minimum contact time) and/or autoclaved.
5. ***Routine laboratory cleaning*** will be done by lab personnel within the containment room.

Accidents:

Spills:

Effective disinfectants (10% bleach, Wescodyne or Quatricide PV Solution) will be made available in the laboratory at all times and for immediate use. In the event of a spill or container breakage resulting in the unintentional release of a biological agent:

(i) Place bleach soaked paper towel or absorbent on the liquid
(ii) Pour a strong disinfectant solution (i.e. use same product used to soak the paper towel in (i), do not mix different agents together) around, but not on the spill, and mix the disinfectant with the spilled material cautiously;
(iii) Evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material, normally 20 minutes;

(iv) Carefully place absorbent paper into a bag for incineration;
(v) Decontaminate all surfaces exposed to the spill with the appropriate disinfectant allowing for appropriate contact times.

If aerosols may have been created in the spill or unintentional release, evacuate the laboratory for a time sufficient for most aerosols to settle, be dispersed, or removed by the ventilation system, usually 20-30 minutes. The use of respiratory protection should be considered for re-entry. Then proceed with items (i)-(v) above. During an emergency, the first priority is the protection of the health and safety of personnel, followed by the environment (i.e. sewer drains), followed by equipment or property.

Spills within a biological safety cabinet

- Leave the ventilation on
- All items within the cabinet should be disinfected (Walls and surfaces wiped down, equipment wiped down and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Pour a strong disinfectant solution (i.e. use same product used to soak the paper towel in (i), do not mix different agents together) around, but not on the spill, and mix the disinfectant with the spilled material cautiously; Leave on for 20 to 30 minutes
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Ventilation should run 10-15 minutes before continuing work in BSC

Spills within an incubator

- All shelves and walls within the incubator should be disinfected (walls and surfaces wiped down, and/or autoclaved)
- Cover the spill area with paper towels or absorbent material
- Soak the spill area with an appropriate disinfectant (i.e.Wescodyne, or Quatricide PV Solution) Pour the disinfectant from the outside surface of the absorbent material towards the inside, surrounding the spill. Leave on for 20 to 30 minutes (close the door of the incubator during the disinfection time)
- Pick up with absorbent material and place in biohazard bag to be then autoclaved
- Finish by wiping the incubator with 70% ethanol

First Aid:

In the case of any incident or accident, personnel must seek medical treatment and notify the Principle Investigator or Laboratory Supervisor. An accident/incident reporting form must be completed:
http://www.uwo.ca/humanresources/facultystaff/h_and_s/acc_inc/accident_inc_index.htm

Eye exposure from splash or aerosol:

Rinse a minimum of 15 minutes in eye wash or flush with water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Skin Exposure or Abrasions:

Contaminated skin or abrasions should be scrubbed with germicidal soap and copious amounts of water. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Inhalation:

Remove person to fresh air. Notify the Principal Investigator or Laboratory Supervisor, who will immediately contact Workplace Health at 519-661-2047 and direct the exposed employee to appropriate medical treatment and to report the incident.

Gunaratnam.

------- Original Message --------
Subject: Re: Containment Level - Lentiviral project
Date: Fri, 23 Apr 2010 09:35:57 -0400
From: Permit-Permis <permitpermis@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer Stanley
Thank you for contacting our Directorate with your questions.
I regret that the Office of Laboratory Security has a policy of not undertaking risk assessments of the human pathogens in facility's inventories, as many of the factors that come into play are specific to a particular location and application. The determination of what risk group to which your pathogens belong is your responsibility.
As a first means of assisting you in determining your inventory's risk groups(s), you may want to consult the schedules for Risk Groups 2, 3, and 4 that are appended to The Human Pathogens and Toxins Act. Schedules 2 to 4 of the Act provide, respectively, non-exhaustive lists or examples of the kinds of human pathogens that are included in each of risk groups 2, 3, and 4. See link below:
http://www2.parl.gc.ca/HousePublications/Publication.aspx?Docid=4015133&file=4
If you possess human pathogens that are not included in these schedules, then you would have to determine the risk groups of those pathogens yourself. First, you could consult the definitions of the Risk Groups that are provided in section 3 of the Act. Further, there is greater detail on the criteria for determining the risk group of a pathogen in sections 2.1, 2.3 and 7.2 of the Laboratory Biosafety Guidelines . See attachment below:

For more information you can visit our website;
http://www.phac-aspc.gc.ca/ols-bsl/pathogen/index-eng.php

Regards

Josee Davies
Regulatory Technologist/ technologiste en réglementation
Pathogen Regulation Directorate (formerly Office of Laboratory Security) /
Direction de la réglementation des agents pathogènes (anciennement le Bureau de la sécurité des laboratoires)
Public Health Agency of Canada/ Agence de santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A Ottawa, Ontario, Canada K1A 0K9
Tel: (613) 957-1779
Fax: (613)941-0596

Jennifer Stanley <jstanle2@uwo.ca> 2010-04-22 02:53 PM
To Permit-Permis permitpermis@phac-aspc.gc.ca cc
Subject Containment Level - Lentiviral project

Hello there,
Please let me know what containment level you suggest for the following project:

Lentiviral technology will be used to introduce or silence (SiRNA/shRNA) our gene of interest, KIM-1, into established or primary cells in culture. We are planning on purchasing Lentivirus for SiRNA/shRNA from Santa-Cruz Biotechnology: http://datasheets.scbt.com/sc-61691-sh.pdf
Expression vectors will be purchased from Clonetech:
http://tools.invitrogen.com/content/sfs/manuals/virapower_lentiviral_system_man.pdf

Lentivirus technology will be used to express KIM-1 or siRNA/shRNA targeting KIM-1 in mouse primary cells and cell lines that are not easily transfectable at high efficiency. The cell types to be infected with lentivirus are: primary mouse cells (kidney and T cells) and ATCC cell lines HEK 293, LLC-PK-1, 769-P, 786-0 and CMT-93.

Regards,

Jennifer

Name of Candidate

Lakshman Gunaratnam

Project Title: **Mechanism of Negative Regulation of Danger Signaling and Kidney Transplant Rejection by Kidney Injury Molecule-1**

Abstract

Overview

Kidney transplantation remains the treatment of choice for patients with end-stage renal disease. One of the major problems in transplantation is immunologic rejection of the graft. As T cells are both required and sufficient for rejection of allotransplants, transplantation immunology research has largely focussed on the adaptive immune system. An increasing body of evidence suggests a key role for the innate immune response in activating dendritic cells (DCs) that ultimately direct allogeneic T cells to mediate allograft rejection. Release of endogenous "danger" signals by damaged tissue or necrotic allograft cells due to ischemia reperfusion injury (IRI) is a powerful activation signal for DCs. Kidney injury molecule-1 (KIM-1) is a novel scavenger receptor that is highly upregulated in renal tubular epithelial cells (RTECs) and converts them into phagocytes for apoptotic and necrotic cells. We hypothesize that upregulation of KIM-1 allows RTECs to inhibit allograft rejection by negatively regulating activation of DCs by HMGB-1, a key "danger" signal released by RTECs following IRI. By uncovering how RTECs regulate innate immunity, we hope to identify therapeutic strategies to prevent transplant rejection and improve overall graft survival in transplant patients.

Background

DCs like other innate immune cells use pattern recognition receptors (PRRs) to recognize conserved molecules termed pathogen-associated molecular patterns (PAMPs) on bacteria and other evolutionarily distinct organisms. Ligation of PRRs (e.g. Toll-like receptors, TLRs) on innate immune cells by PAPMs triggers a cascade of signalling events that lead to production of pro-inflammatory cytokines, adhesion molecules, chemokines and antimicrobial peptides that allow them to function as a fist line of defence against pathogens. Alloreactive T cells can recognize intact donor MHC molecules on "passenger" (present within the organ before harvesting) antigen presenting cells (APCs) (*1*) or, indirectly, when recipient APCs capture, process and present donor MHC and non-MHC alloantigens to recipient T cells in a host-MHC restricted fashion (*2*).(*3*). Engagement of naïve T cells by APCs in the absence co-stimulatory signals results in T cell anergy or apoptosis. Maturation of resting DCs to express co-stimulatory molecules can be triggered by binding of pathogens (or PAMPs) to PRRs on their surface. One of the vexing questions in transplant immunology is how naïve T cells become activated in the absence of pathogenic stimuli (e.g. PAMPs), especially, given that transplant surgery for the most part is a sterile procedure.

Work done by Matzinger and others, suggests that PRRs can also be activated by host-derived "danger" or "alarm" signals sent by injured, damaged or necrotic cells (*4-5*). A number of endogenous "danger" or damage associated molecular patterns (DAMPs) have been identified including high-mobility group B-1 (HMGB1), heat shock protein 70 (hsp70), hyaluronan (HA) and S100s (*6*). HMGB1, is a ubiquitously expressed and highly conserved chromatin-associated protein is released extracellularly by necrotic cells, but not by apoptotic cells (*7*) (*8*). HMGB1 can also be secreted by activated macrophages, DCs and NK cells or non-immune cells in response certain inflammatory stimuli such as LPS, IFN-γ or interleukin-1 (IL-1) (*9*). Binding of extracellular HMGB1 to its receptor(s), the receptor for advanced glycation end products (RAGE) and members of TLR family (i.e. TLR-2, TLR-4, and TLR-9), triggers several signalling pathways in target cells including MAPK and nuclear factor-κB (NF-κB), which in DCs, can trigger upregulation of co-stimulatory molecules, enhance antigen presentation via MHC class II and secretion of pro-inflammatory cytokines (e.g. IL-12, IL-6, IL-1β, IL-8, TNF-α, and RANTES) (*9-10*).

RTECs are a major target of IRI and often undergo apoptosis or even necrosis as a result (*11-17*). The importance of IRI in the regulation of T cell-mediated allograft rejection is highlighted by the fact that "parking" cardiac allografts in immunodeficient hosts to allow them to heal from IRI prevented acute rejection when grafts were exposed to active alloreactive T cells later on (*18*). Recent evidence has now implicated HMGB1 as an early mediator of inflammation in hepatic IRI as administration of neutralizing antibody to HMGB1 significantly decreased liver injury (*19*). These and several other studies together suggest that danger signals from injured (IRI) allografts may play a central role in priming naïve alloreactive T cells and promote rejection by stimulating DCs (*20-21*).

KIM-1 is a novel scavenger receptor protein that is highly upregulated *in vivo* on the surface of proximal RETCs within hours of IRI or after renal transplantation (*22-25*). Recently a number of groups including Bonventre's, demonstrated that KIM-1 binds to phosphatidylserine (PS), an "eat-me" signal exposed on the surface of cells undergoing apoptosis and can mediate phagocytosis of apoptotic and necrotic cells (*26-28*). *In vivo*, the remnants of apoptotic and necrotic cells can be visualized within phagosomes of KIM-1-expressing RTECs in rodent models of AKI (*23, 27, 29*). The exact physiologic role of KIM-1 in RTECs *in vivo* remains unknown.

Preliminary Data

Mice engineered to express phagocytosis-defective mutant KIM-1 are more susceptible to IRI and accumulate apoptotic and necrotic cells within the injured tubular lumen of kidneys (Bonventre, personal communication). Data presented in figure 1 suggests that HMGB1 can be detected in the urine of mice at least within twelve hours after IRI but not in sham treated mice. As HMGB1 is released from necrotic cells, we exposed KIM-1 expressing LLC-PK1 cells in culture to necrotic cells and measured residual extracellular HMGB1 in the conditioned medium after 24 hours to test if KIM-1 regulates the availability of this extracellular danger signal (Figure 2). The conditioned medium from KIM-1 expressing cells had significantly less extracellular HMGB1 compared to non-KIM-1 expressing controls suggesting that KIM-1 may play a role in regulating secretion and or removal of extracellular HMBG1 (or necrotic cells).

Rationale and Hypothesis

KIM-1 is highly upregulated in RTECs during IRI and that it is a scavenger receptor capable of mediating phagocytosis of necrotic cells. Together with the preliminary data presented here, we propose that HIM-1 may regulate danger signalling following IRI in renal transplantation.

Hypothesis: KIM-1 negatively regulates allogeneic Tcell immunity to kidney allografts by sequestering HMGB1 and blocking danger signalling to DCs.

Specific Aims and Experimental Plan

Specific Aim 1: To determine the mechanism by which KIM-1 regulates extracellular HMGB1. ***Rationale:*** Elucidating the exact molecular mechanism of how KIM-1 regulates HMGB1 availability may help us design experiments to specifically block this pathway in animal models and moreover uncover strategies to enhance this mechanism in a clinical setting. It would be important to distinguish whether KIM-1 is preventing release of HMGB1 by RTECs exposed to necrotic cells or by actively removing it from the extracellular medium. We have thus designed experiments to test the following mechanisms: First goal: Determine whether KIM-1 binds directly to HMGB1 (primary sequestration) and degrades it via receptor-mediated endocytosis. *Second goal:* Determine if passive release of HMGB1 is inhibited by KIM-1-dependent phagocytosis of necrotic cells (secondary sequestration). Third goal: Determine if KIM-1 expression on RTECs blocks HMGB1 release (from RTECs) when they are exposed to necrotic cells.

Methodologies: In order to test our hypotheses, we will use a variety of RTEC types such as LLC-PK1 cells, MDCK cells as well as primary mouse RTECs and non-tubular HEK293 cells. The basic assay will consist of exposing RTECs to either to no treatment, live cells, necrotic cells or recombinant HMGB1 (added to medium after monolayer has formed rHMGB1), allowing time for uptake or release, and then measured extracellular HMGB1 in the conditioned medium by western blotting and/or ELISA. To test whether KIM-1 binds to HMGB1, we will immunoprecipitate either molecule after exposing KIM-1-expressing RTECs to rHMGB1 or necrotic cells and then detecting the interaction by Western blot. To determine if internalized HMGB1 is degraded by RTECs, we will simultaneously treat cells with proteasome or lysosomal inhibitors before adding necrotic cells and then detecting the interaction by Western blot. To determine if bound HMGB1 is degraded we will simultaneously treat cells with proteasome or lysosomal inhibitors in the above experiment. Necrotic cells will be generated by exposing either Jurkat cells (ATCC) or primary mouse splenocytes to multiple freeze-thaw cycles (*5*).

Specific Aim 2: To determine whether KIM-1-epressing RTECs inhibit danger singling by extracellular HMGB1 and inhibit activation of DCs.

Rationale: HMGB1 can function as an adjuvant to stimulate allogeneic T cell immunity by activating DCs (danger signalling) by inducing phenotypic maturation of DCs (*10, 30*). First goal: Determine how conditioned medium from KIM-1-expressing RTECs after exposure to necrotic cells or rHMGB1 affects DC activation *in vitro*. Second goal: Determine how KIM-1-expressing RTECs co-cultured with DCs exposed to necrotic cells or

rHMGB1 (simultaneously) affect DC activation *in vitro*. Third goal: Determine how DC activated as above (first and second goals) affect allogeneic T cell responses (direct allorecognition).

Methodologies: We will use primary mouse (SCID) bone marrow-derived DCs (*5*) or widely available DC lines (e.g. ATCC-CRL-11904). RTECs will be cultured in monolayers in transwell plates with DCs or conditioned medium will be transferred from RTECs after exposure to necrotic cells or rHMGB1. For T cell activation assays, DCs (from C57BL/6 vs. DBA/2 mice) will be treated with as above (first and second goals) and then co-cultured with primary CD4 T cells isolated from mice or allergenic T cell clones before measuring proliferation using ^{3}[H]-thymidine or CFSE dye assays as described (*10*). Quantitative RT- and ELISA will be used to measure release of proinflammatory cytokines such as IL-12, IL-6, IL-1alpha, IL-8, TNF-alpha, and RANTES. Phenotypic maturation of DCs (after treatment as above) will be measured by flow cytometric analysis for increased surface markers such as CD83, CD54, CD80, CD40, CD58, and MHC class II and decreased CD206 expression . Alternatively ELISPOT can be used to measure T cell reactivity (*31*).

Specific Aim 3: To test if targeted deletion of kim-1 in murine kidney allografts exacerbates danger signalling and promotes allograft rejection. *Rationale:* HMGB1 (Figure 1) and KIM-1(*29*) can be detected in the urine of rodents subjected to IRI. In vitro studies often oversimplify complex regulatory pathways and may not represent finding *in vivo*. This aim will test if KIM-1 negatively regulates allograft rejection *in vivo*. First goal: Monitor survival, renal function and urinary KIM-1/HMGB1. Second goal: Perform histological and functional analysis on kidney tissue, DCs and T cells.

Methodologies: C57BL/6, H2-2^{b} kidneys will be transplanted into recipient (DBA/2, H-2^{d}) mice as described (by Noris et al.) by Dr. Zhang (our core veterinary surgeon). Syngeneic transplants will be used to monitor for non-alloimmune effects on the graft while sham treated mice will serve as naïve controls. Parallel groups of experiments will be performed. Mice in one group will be sacrificed on days 1,3, 6 and 12 and kidney grafts will be divided and used to perform: histological analysis (tubular injury, tubulitits, leukocyte infiltration, atrophy, total tubulointerstitial injury score), measure urinary KIM-1/HMGB1, in situ hybridization (KIM-1), RT-PCR (TLR-2, TLR4, TLR9, RAGE, TNF-α, MIP-2, MCP-1, IL-1β, IL-6), immunostaining (macrophages, granulocytes, T cells, B cells, DCs [CD11b+ and CD11c+], HMGB1, KIM-1, MHC-II [H-2^{b} and H-2^{d}]). We will also examine sections for total CD8+, CD4+, CD4+FoxP3 T cells per field. In the second group we will monitor survival, and measure serum BUN, creatinine, urinary KIM-1/HMGB1 at on odd days for 2 weeks and then at 30 and 60 days. We will measure recipient T cell alloreactivity exposing recipient splenocytes to irradiated donor (H-2^{b}) or third-party (DBA/1, H-2^{q}) splenocytes or isolated DCs (bone marrow or from the allograft). Sham treated mice will serve as additional controls in all experiments.

Expected Results:

We predict that KIM-1 expressed on injured RTECs decreases available HMGB1 by removing necrotic cells from the extracellular milieu (indirect sequestration). RTECs expressing mutant KIM-1 that cannot bind phosphatidylserine (data not shown) should behave like non-KIM-1-expressing cells. Given that HMGB1 and necrotic cells can activate DCs (*3, 5*), we expect that transfer of conditioned medium from KIM-1-expressing RTECs will stimulate DCs to a much lesser degree than non-KIM-1-expressing cells. This effect should translate into reduced allostimulatory effect of DCs activated by conditioned medium from KIM-1-expressing RTECs. Unless, KIM-1-expressing RTECs have a direct dominant positive effect on DCs after exposure to HMGB1, simultaneous co-culture experiments (specific aim 1) are unlikely to yield any differences in DC activation regardless of KIM-1 status. Finally, we do expect that mice that receive KIM-1-deficient kidneys will have exhibit higher incidence of rejection. We also expect that there will be a detrimental effect from KIM-1 deficiency even in the syngeneic recipients.

Where Funding Will Be Applied For:

1. Canadian Institutes of Health Research: Operating Grant (09/2010 deadline)
2. Kidney Foundation of Canada: Biomedical Research Grants (15/10/2010 deadline)
3. Natural Sciences and Engineering Research Council: Discovery Grant (08/210 deadline)

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1. Purpose:

To describe the biological safety risks of working with lentiviral vectors and the engineering and operational practices that are approved for mitigating these risks. Note that these are minimum standards and that some experiments may require higher containment practices. This should be decided jointly by the Principal Investigator and the University Biohazard Committee.

2. Applicable Legislation, Standards, Guidelines:

Public Health Agency of Canada Laboratory Biosafety Guidelines, 3rd edition, 2004
Canadian Food Inspection Agency Veterinary Standards for Animal Facilities
National Institutes of Health, Recombinant DNA Advisory Committee, Guidance on Biosafety Considerations for Research with Lentiviral Vectors (March 2006)
http://www4.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/index.htm

3. Requirements:

No one is allowed to work with lentivirus without having prior training by the Principal Investigator who supervises their work, or their designated technical expert. The worker should demonstrate good microbiological and tissue culture technique and an understanding of this SOP prior to being permitted to work with lentivirus.

The use of or generation of any new lentiviral constructs must to be cleared with the Principal Investigator.

The use or generation of any new lentiviral construct that involves a different transgene or a different type of alteration than that already approved by the Biohazard Committee (e.g. the knock-out rather than the expression of a particular protein, its subunits or its mutants) must be communicated to the Biohazard Committee via an application for an amendment to the laboratory's Biohazard Permit. Constructs that involve different mutations in a particular protein, where the work on that protein has already been approved, do not require an amendment as long as the Principal Investigator is confident that no additional biohazard risk is being created.

4. Biological Safety Risk:

- Lentiviral particles are usually pseudotyped with the vesicular stomatitis virus glycoprotein (VSVG), an envelope protein which gives the virus the ability to infect many human and mammalian cell types. The skin affords some protection, but the virus may enter the body through chapped skin or wounds or by infecting mucosal surfaces (eyes, nose, mouth).

Pseudotyping with other proteins will change the tropism of the viral particle and therefore alter the risk.

- Lentivirus is an enveloped virus, so it is susceptible to inactivation by sufficiently long treatment with 70% ethanol or freshly diluted 10% bleach.
- The direct effect on the infected cell will depend in part on the protein that the virus is engineered to produce (the product of the transgene).
- Lentivirus can integrate into chromatin in non-dividing cells.
- Lentivirus integrates into chromatin (randomly) so there is an unpredictable risk associated with gene disruption. Note that some gene disruptions may promote abnormal cell growth.
- Lentiviral vectors are engineered to be replication incompetent so that the infection will not spread beyond the site of infection. However there is a risk of generating infective replication competent virus through recombination. Later generation vectors in which 3 or 4 plasmids are used to produce the viral particles and which a deletion is created in the LTR upon integration, have a lower risk of generating replication competent virus, so their use is encouraged where practical.
- Lentiviral vectors are generally classed as biohazard risk group 2. However they may be classed as risk group 2+ and require additional operational precautions if the vector system is an earlier generation, or if the transgene encodes a biological toxin, an oncogene, a cell cycle regulator or an inhibitor of a tumor suppressor (eg. siRNA for a tumor suppressor). If this is the case it will be indicated on the biohazard permit approved by the Queen's Biohazard Committee.
- Lentivirus cannot replicate in rodents, but because rodents can shed virus for days after infection, infection of rodents must occur at containment level 2. If the animals have been transplanted with human cells, then if replication competent virus is generated then it could replicate in the transplanted cells.

5. Biohazard Containment Facilities and Procedures:

- All work with lentivirus should be carried out in a containment level 2 laboratory. Lentivirus in supernatants and cells must be inactivated before removal to lower containment. Inactivation must be by an approved method (eg. 0.5% Triton-X 100 extraction of cells at 37°C for 30 min.).
- If cells are to be transported to another level 2 containment laboratory approved for lentiviral work, a secondary container with a tight fitting lid to prevent spills must be used for transport.
- If cells are infected that would normally only require level 1 containment, then the cells can be removed from level 2 containment after demonstrating that there is no longer any infective virus in the culture supernatant. This often requires three or four passages (not simply washing). Demonstration of the absence of lentivirus in the culture supernatant may be done using a p24 ELISA, with the supernatant from the day of infection used as a positive control

and from uninfected cells as a negative control. In addition to demonstrating the destruction of the input viruses, this is necessary to exclude the possible, although rare, development of a replication competent virus.

- Virus will be shed from rodents infected with lentivirus for a few days after infection, during which time the animals must be held at containment level 2. After the cage and bedding have been changed and shedding of virus has ceased (as demonstrated by an ELISA for p24 antigen or other approved method) then the animals may be moved to containment level 1. However, if the animals have been transplanted with human cells, then there is a risk that the virus could replicate in the transplanted cells and the animals must be handled at containment level 2.
- Unless higher containment practices are required by the Queen's Biohazard Committee, strict biosafety level 2 operational practices should be employed for manipulations of lentivirus. Review the Public Health Agency of Canada Laboratory Biosafety Guidelines, chapter 3 (3rd edition, 2004) before beginning work with lentivirus. **In particular, the following points are reinforced:**
  - Gloves should be worn during all tissue culture manipulations. Double gloves are better because micro-holes may be present. Inspect gloves for obvious holes when you are putting them on. Change outer gloves at regular intervals and whenever they become obviously contaminated. Wash your hands thoroughly with soap immediately after removing gloves. Lab coats with knit cuffs are recommended to ensure that no bare skin is exposed between the gloves and the lab coat.
  - Always treat your outer gloves as contaminated – remove them and replace with clean gloves before touching things outside of the biological safety cabinet (e.g. the microscope, the centrifuge, or the incubator); if necessary use the one clean hand / one dirty hand technique.
  - People often touch their face and eyes unconsciously. If you find that you are doing this with gloves on your hands then wear goggles to prevent yourself from doing so since this could result in you infecting yourself.
  - All work must be done in a biological safety cabinet so that the virus is not spread via aerosols. Use proper technique, not over-filling the cabinet and not putting anything on the front grill because this will disrupt the air flow, reducing your protection and potentially resulting in contamination of your cultures.
  - Centrifugation must take place in screw capped tubes (including microfuge tubes). Do not over-fill the tubes and do decontaminate their outer surface with 70% ethanol or quaternary ammonium disinfectant before removing them from the biological safety cabinet. Aerosol resistant centrifuge cups that are opened only in the biological safety cabinet are recommended, but not strictly required.
  - The garbage containing lentivirus contaminated dishes, filters, syringes, gloves etc. should be collected inside the biological safety cabinet. Immediately after completing work, seal it into a second autoclave bag and take it to the autoclave for disinfection. Unseal for autoclaving to allow steam penetration.

- Avoid the use of sharps whenever possible and dispose of them immediately in a sharps container within the biological safety cabinet. If feasible use plastic disposable transfer pipettes rather that glass Pasteur pipettes. Eliminate the use of needles whenever possible or use safety engineered needles. Use disposable scalpels and safety engineered scalpels.
- Glass serological pipettes and Pasteur pipettes should be immediately submerged into jars filled with freshly diluted bleach (1:10 dilution of bleach) inside of the biological safety cabinet. After 30 minutes in bleach, Pasture pipettes may be transferred to the glass disposal container and serological pipettes moved to a bucket for washing. If space constraints make it seem unreasonable to decontaminate serological pipettes in the biological safety cabinet, then discuss alternatives with the University Biosafety Officer.
- The plastic tubing and aspiration bottle should be disinfected immediately after use by drawing concentrated bleach through the line and into the collection bottle. Allow the bottle to sit for 30 minutes for full decontamination after the last addition before discarding the liquid in the sewer. After emptying the collection bottle add sufficient bleach that a 10% dilution will be achieved when the bottle is full. Be sure that the vacuum is protected with a HEPA filter.
- Liquid spills on any surfaces should be immediately disinfected with diluted bleach (30 minute contact time). Spills that are allowed to dry are much more difficult to decontaminate and must be rehydrated first. Be sure to rinse off the bleach well from stainless steal because it will corrode.
- The dishes containing lentivirus should be put in a secondary container to move from the biological safety cabinet to the incubator to reduce the risk of spills. They should be kept in a designated incubator whenever possible. Take care not to contaminate the door handle of the incubator.
- When finished work, decontaminate the outer surface of everything that is in the biological safety cabinet and then remove all items from the cabinet. Wash the hood thoroughly with 70% alcohol or other approved disinfectant. The use of UV light to disinfect the hood is not recommended because it is not effective unless properly maintained and because it can present a UV exposure hazard to other users of the laboratory unless the cabinet has a sash that can be completely closed.
- Ensure that the appended reminder sheet is posted in the laboratory where lentivirus is used.

6. First Aid:

- Following a splash or accidental touching of the eyes, nose or mouth with material potentially contaminated with lentivirus, **immediately** flush the eyes or other mucosal surfaces at an

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eyewash station for **15 minutes**. Remember to remove your gloves before using your fingers to keep your eyes open.

- Wear double gloves to protect broken skin. A wound or broken skin (eg. chapped, eczema) potentially contaminated with lentivirus should be **immediately** washed with soap and running water for 15 minutes with gentle massaging.
- Seek follow-up medical attention if required.
- Report the incident to your supervisor and have them fill in an incident report (WSIB form 7) which is to be submitted to the Department of Environmental Health and Safety within 24 hours of the incident.

7. **Information and Enquires:** University Biosafety Officer (613-533-6000 ext. 77077)

8. **Revision History:**

Initial release: March 2008

ATCC

MATERIAL SAFETY DATA SHEET

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I
Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
Physical data

Pink or red aqueous liquid

SECTION III
Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. 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.

SECTION IV
Fire and explosion

Not applicable

SECTION V
Reactivity data

Stable. Hazardous polymerization will not occur.

SECTION VI
Method of disposal

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII
Special protection information

For Biosafety Level 1 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII
Special precautions or comments

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** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

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Cell Biology

ATCC® Number: CCL-34™ Order this Item
Price: $269.00

Designations: MDCK (NBL-2)

Depositors: S Madin, NB Darby

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Canis familiaris*

Morphology: epithelial

PHOTO

Source: **Organ:** kidney
Disease: normal

Cellular Products: 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 matenal. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Isolation: **Isolation date:** September, 1958

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

Virus Susceptibility:
Human Coxsackievirus B 5
Reovirus type 2
Adeno-associated virus 4
Vaccinia virus
Vesicular stomatitis virus
Adeno-associated virus 5
Human Coxsackievirus B 3
Human Coxsackievirus B 4
Human poliovirus 2

Reverse Transcript: negative

Cytogenetic Analysis: Polyploidy 0.2%. Two large submetacentric chromosomes noted, presumably X chromosomes, and one or two additional chromosomes with median or submedian centromeres.

Age: adult

Gender: female

Comments: The MDCK cell line was derived from a kidney of an apparently normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby.The cells are positive for keratin by immunoperoxidase staining.MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its proteolytic products.

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Cell Biology

ATCC® Number: CL-101™ Order this Item
Price: $275.00

Designations: LLC-PK1
Depositors: Eli Lilly & Co.
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Organism: Sus scrofa (pig)
Morphology: epithelial
Source: **Organ:** kidney
Strain: Hampshire
Disease: normal
Cellular Products: plasminogen activator
Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Age: 3 to 4 weeks
Gender: male
Propagation: **ATCC complete growth medium:** The base medium for this cell line is Medium 199 containing 1.5 g/L sodium bicarbonate. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 3%.
Temperature: 37.0°C
Subculturing: **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: Twice per week
Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach.
Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation: culture medium 95%; DMSO, 5%
Related Products: recommended serum:ATCC 30-2020
formerly distributed as:ATCC CRL-1392
References:
3520: Hull RN, Huseby RM. Enhanced production of plasminogen activator. US Patent 3,904,480 dated Sep 9 1975
22659: Perantoni A, Berman JJ. Properties of Wilms' tumor line (TuWi) and pig kidney line (LLC-PK1) typical of normal kidney tubular epithelium. In Vitro 15: 446-454, 1979. PubMed: 225262
28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321

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Cell Biology

ATCC® Number: CRL-1573™ Order this Item
Price: $256.00

Designations: 293 [HEK-293]
Depositors: FL Graham
Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Homo sapiens* (human)
Morphology: epithelial

PHOTO

Source:
Organ: embryonic kidney
Cell Type: transformed with adenovirus 5 DNA

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

Restrictions: These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications:
efficacy testing [92587]
transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
viruscide testing [92579]

Receptors: vitronectin, expressed

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis: 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.

Age: fetus

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Cell Biology

ATCC® Number:	CRL-11268™ Order this Item
Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
Age:	fetus
Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.

Price: $272.00

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ATCC® Number: TIB-152™ Order this Item **Price:** $272.00

Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
	PHOTO
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-13,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.
Gender:	male

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ATCC® Number:	CRL-11904™ Order this Item	**Price:**	$289.00
Designations:	JAWSII		
Depositors:	ZymoGenetics, Inc.		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	mixed, adherent and suspension		
Organism:	*Mus musculus* (mouse)		
Morphology:	monocyte PHOTO		
Source:	**Organ:** bone marrow **Strain:** C57BL/6 **Cell Type:** immature dendritic cell; monocyte;		
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.		
Propagation:	**ATCC complete growth medium:** Alpha minimum essential medium with ribonucleosides, deoxyribonucleosides, 4 mM L-glutamine, 1 mM sodium pyruvate and 5 ng/ml murine GM-CSF, 80%; fetal bovine serum, 20% **Temperature:** 37.0°C		
Subculturing:	**Protocol:** Cultures can be maintained by transferring floating cells to a centrifuge tube. Attached cells may be subcultured using 0.25% trypsin-0.03% EDTA. Pool cells and centrifuge the cell suspension at 1000 rpm for 10 minutes, resuspend the pellet in fresh medium, aspirate and dispense into new flasks. Note: This cell line grows very slowly. **Subcultivation Ratio:** A subcultivation ratio of 1:2 is recommended **Medium Renewal:** Once per week		
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase		
Related Products:	recommended serum:ATCC 30-2020		
References:	38868: MacKay VL, Moore EE. Immortalized dendritic cells. US Patent 5,648,219 dated Jul 15 1997 47440: Moore EE. Preparation of immortalized cells. US Patent 5,830,682 dated Nov 3 1998		

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Cell Biology

ATCC® Number:	**CRL-2190™** Order this Item
Designations:	HK-2
Depositors:	RA Zager
Biosafety Level:	2 [Cells Contain Papilloma viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney, cortex **Tissue:** proximal tubule **Cell Type:** human papillomavirus 16 (HPV-16) transformed
Cellular Products:	alkaline phosphatase; gamma glutamyltranspeptidase; leucine aminopeptidase; acid phosphatase; cytokeratin; alpha 3, beta 1 integrin; fibronectin
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:	epidermal growth factor (EGF), expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 13 D13S317: 9 D16S539: 11,12 D5S818: 12 D7S820: 10,11 THO1: 9 TPOX: 8,9 vWA: 17,18
Age:	adult
Gender:	male

Price: $272.00

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Cell Biology

ATCC® Number: CRL-2123™ Order this Item
Price: $349.00

Designations: mIMCD-3

Depositors: S Gullans

Biosafety Level: 2 [CELLS CONTAIN PAPOVAVIRUS]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: Mus musculus, transgenic (mouse, transgenic)

Morphology: epithelial

Source:
Organ: kidney, medulla
Tissue: collecting duct
Cell Type: SV40 transformed

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

Isolation: **Isolation date:** 1991

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Age: adult

Comments: mIMCD-3 is an inner medullary collecting duct (IMCD) cell line derived in 1991 by Michael Rauchman from a mouse transgenic for the early region of SV40 [Tg(SV40E)bn/7].
A tubule from the terminal one-third of the IMCD was microdissected and placed in culture.
Confluent cells were subcultured and cloned using cloning cylinders.
This is a polarized epithelia cell line which retains many differentiated characteristics of the terminal IMCD including inhibition of apical to basal sodium flux by amiloride and by atrial natriuretic peptide (ANP).
The cells possess an amiloride sensitive sodium channel as determined by Western blot analysis, and accumulate the major organic osmolytes (inositol, sorbitol, betaine and glycerophosphorylcholine) in response to hypertonic stress.
The cells secrete endothelin and form tubules and tight junctions.
mIMCD-3 cells are responsive to Hepatocyte Growth Factor (HGF), and are readily adaptable to growth in hypertonic medium supplemented with NaCl and urea up to 910 mosmol/kg H20.
These extreme osmotic conditions exist in the renal medulla in vivo, but are known to be lethal to most other cells.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated DMEM:F12 Medium Catalog No. 30-2006. 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
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

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ATCC® Number:	**CRL-1932™** Order this Item **P**
Designations:	786-O [786-0]
Depositors:	RD Williams
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney **Disease:** renal cell adenocarcinoma
Cellular Products:	parathyroid hormone (PTH) like peptide
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulat permits may be required for the transfer of this ATCC material. Any purchasing ATCC material is ultimately responsible for obtaining permits. Please click here for information regarding the spe requirements for shipment to your location.
Applications:	transfection host (technology from amaxa)
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10 D13S317: 8 D16S539: 12 D5S818: 9 D7S820: 11,12 THO1: 6,9.3 TPOX: 8,11 vWA: 15,17
Cytogenetic Analysis:	hypertriploid; Y was present in 60% the cells examined
Age:	58 years
Gender:	male
Ethnicity:	Caucasian

Product Description

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ATCC® Number:	**CCL-223™** Order this Item	**Price:**	**$329.00**
Designations:	CMT-93		**Related Links**
Depositors:	LM Franks		NCBI Entrez Search
Biosafety Level:	1		Make a Deposit
Shipped:	frozen		Frequently Asked Questions
Medium & Serum:	See Propagation		Material Transfer Agreement
Growth Properties:	adherent		Technical Support
Organism:	*Mus musculus* (mouse)		Related Cell Culture Products
Morphology:	epithelial		
Source:	**Strain:** C57BL/icrf **Organ:** rectum **Disease:** polyploid carcinoma		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Tumorigenic:	Yes		
Reverse Transcript:	positive		
Antigen Expression:	H-2b		
GenoType:	a(t)		
Age:	19 months		
Gender:	male		
Comments:	Tested and found negative for ectromelia virus (mousepox).		
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%. **Temperature:** 37.0°C		

SANTA CRUZ BIOTECHNOLOGY, INC.

TIM-1 siRNA (h): sc-61691

BACKGROUND

CD4+ T helper lymphocytes can be divided into types 1 (Th1) and 2 (Th2) on the basis of their cytokine secretion patterns. Th1 cells and their associated cytokines are involved in cell-mediated immunity to intracellular pathogens and delayed-type hypersensitivity reactions. Th2 cells are involved in the control of extracellular helminthic infections and the promotion of atopic and allergic diseases. T cell Ig- and mucin-domain-containing molecules (TIMs) are a family of molecules expressed on T cells. TIM-1 is a single-pass type I membrane protein that is associated with the development of Th2 biased immune responses and selectively expressed on Th2 cells. TIM-1, also designated hepatitis A virus cellular receptor-1 (HAVcr-1) or T cell membrane protein 1, acts as a cell-surface receptor for hepatitis A virus and may also play a role in asthma and allergic disease regulation. TIM-1 is a widely expressed protein with highest levels detected in testis and kidney.

REFERENCES

1. Feigelstock, D., et al. 1998. The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. J. Virol. 72: 6621-6628.
2. McIntire, J.J., et al. 2003. Immunology: hepatitis A virus link to atopic disease. Nature 425: 576.
3. de Souza, A.J., et al. 2005. T cell Ig and Mucin 1 (TIM-1) is expressed on *in vivo*-activated T cells and provides a costimulatory signal for T cell activation. Proc. Natl. Acad. Sci. USA 102: 17113-17118.
4. Mariat, C., et al. 2005. Regulation of T cell dependent immune responses by TIM family members. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 360: 1681-1685.

CHROMOSOMAL LOCATION

Genetic locus: HAVCR1 (human) mapping to 5q33.2.

PRODUCT

TIM-1 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TIM-1 shRNA Plasmid (h): sc-61691-SH and TIM-1 shRNA (h) Lentiviral Particles: sc-61691-V as alternate gene silencing products.

For independent verification of TIM-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-61691A, sc-61691B and sc-61691C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TIM-1 siRNA (h) is recommended for the inhibition of TIM-1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 60 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

TIM-1 (BBCR): sc-80359 is recommended as a control antibody for monitoring of TIM-1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TIM-1 gene expression knockdown using RT-PCR Primer: TIM-1 (h)-PR: sc-61691-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

Lentiviral Expression System

Visit our website for more details! click here

Obtain high-level expression in virtually any cell type with our complete Lenti-X™ Expression System

- **Optimized lentiviral vector and packaging system for high titers and high expression**
- **Transfer genes into dividing and nondividing cells and stem cells**
- **Puromycin resistance allows rapid selection of transduced cells**
- **Safe, replication-incompetent virus**

Recombinant lentiviruses derived from HIV-1 are able to deliver genes into almost any mammalian cell type, including primary cultures, dividing or nondividing cells, and stem cells. Clontech has developed a highly advanced lentiviral expression system that provides the broad cellular tropism of VSV-G pseudotyped lentivirus, high viral titers, and excellent transgene expression. The **Lenti-X Expression System,** which includes the **pLVX-Puro** expression vector and our **Lenti-X HT Packaging System,** enables you to produce exceptionally high titers of safe, replication-incompetent lentivirus from your customized pLVX-Puro vector (Figure 1).

Superior Lenti-X Vectors

Like all our Lenti-X vectors, pLVX-Puro not only carries the LTRs and packaging sequence required for lentivirus production and replication, but it also contains elements that improve transgene expression, titer, and overall vector function. Its WPRE element, believed to promote RNA processing events and nuclear export, imparts a dual benefit (1). First, it acts within the context of viral genomic transcripts to enhance vector packaging and increase the titers of viral supernatants produced from 293T packaging cells. Second, it boosts expression of your gene of interest in target cells by facilitating the production of mature mRNA from transcripts initiated by the vector's internal CMV promoter. Lenti-X vectors also contain a cPPT element that increases nuclear importation of the viral genome during target cell infection, resulting in improved vector integration and more efficient transduction (2).

High-Efficiency Packaging

Our Lenti-X HT Packaging System produces outstanding viral titers due to a synergism of highly optimized components (3). The Lenti-X HT Packaging Mix safely provides all the essential lentiviral packaging and replication gene products *in trans* on a proprietary suite of separate vectors. Selected plasmids in the mixture generate high expression levels for critical viral proteins as a result of Tet-Off® transactivation. For added safety, a split gag-pol gene delivery strategy thoroughly prevents viral replicative functions from being transferred to target cells (3). Finally, the included **Lentiphos™ HT** transfection reagents transfer the Lenti-X HT Packaging Mix, along with your pLVX-Puro vector, into 293T cells with unprecedented efficiency. The resulting high-titer viral supernatants can be used directly, without concentration.

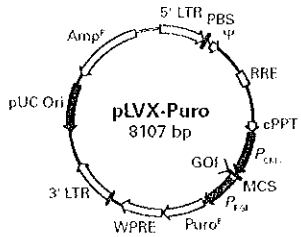


Figure 1. Map of pLVX-Puro. The vector contains the lentiviral-specific LTRs and packaging sequence (Ψ); a multiple cloning site (MCS) to insert your gene of interest (GOI); puromycin resistance; and WPRE and cPPT elements to boost packaging, viral titers, and transgene expression.

High Titers & Rapid Selection

We used the Lenti-X Expression System to generate a high-titer pLVX-Puro supernatant, serial dilutions of which were used to infect naïve cultures of 293T cells (Figure 2). After replating the infected cells on 10 cm dishes and selecting transductants with puromycin, the resulting colonies of stable transductants were stained for detection. Cells infected with only 0.1 µl of supernatant produced hundreds of colonies, while colonies from cells infected with 1 µl virtually covered the entire plate. These results demonstrate the high titer and infectivity of a typical pLVX-Puro supernatant.

Product	Size	Cat. No.	Price
Lenti-X Expression System	each	632164	$1,096.00
Puromycin	25 mg	631305	$71.00
	100 mg	631306	$176.00

Prices are subject to change without notice.

Components

- pLVX-Puro Vector
- Lenti-X™ HT Packaging Mix
- Lentiphos™ HT
- Lenti-X™ Lentiviral Expression Systems User Manual (PT3983-1)

Related Products

- Lenti-X™ Fluorescent Vectors (Cat. Nos. 632152, 632153, 632154 & 632155)
- Lenti-X™ HT Packaging System (Cat. Nos. 632160 & 632161)

Notice to Purchaser

Please see the bGH Poly A, CMV Sequence, cPPT Element, IRES Sequence, Lentiviral Expression Products, Tet-Based Expression Products, VSV-G Technology, and WPRE Technology licensing statements at **www.clontech.com/licensing**

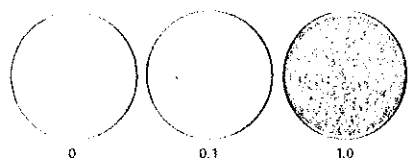


Figure 2. Puromycin selection of transduced cells. 293T cells were infected with the indicated volumes (µl) of pLVX-Puro supernatant and selected with puromycin for 9 days to allow the formation of colonies, which were then stained with crystal violet.

The Lenti-X Expression System is a comprehensive system for preparing recombinant lentivirus to express any cDNA in any cell type susceptible to lentivirus transduction. It easily produces high-titer lentiviral supernatants suitable for safe use with virtually any downstream application.

References

1. Zufferey, R. *et al.* (1999) *J. Virol.* 73(4):2886–2892.
2. Zennou, V. *et al.* (2000) *Cell* 101(2):173–185.
3. Wu, X. *et al.* (2000) *Mol. Ther.* 2(1):47–55.

DAY 1

- Plate target cells in a 12-well plate 24 hours prior to viral infection.
- Add 1 ml of complete optimal medium (with serum and antibiotics) and incubate cells overnight. The cells should be approximately 50% confluent on the day of infection (Day 2).

NOTE: It is possible to use other plate formats for transduction as well. In this case, the amount of cells should be adjusted depending on the growth area of the well or plate.

DAY 2

- Prepare a mixture of complete medium with Polybrene® (sc-134220) at a final concentration of 5 µg/ml.
- Remove media from plate wells and replace with 1 ml of this Polybrene/media mixture per well (for 12-well plate).

NOTE: Polybrene is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined (usually in the range of 2-10 µg/ml). Excessive exposure to Polybrene (>12 hr) can be toxic to some cells.

- Thaw lentiviral particles at room temperature and mix gently before use.
- Infect cells by adding the shRNA Lentiviral Particles to the culture.
- Swirl the plate gently to mix and incubate overnight. The amount of viral particles to use varies greatly depending on the characteristics of the cell line used.

NOTE: Keep thawed shRNA Lentiviral Particles on ice. Repeated freeze-thaw cycles and prolonged exposure of the particles to ambient temperatures may result in decreased viral titers.

NOTE: When transducing a shRNA lentiviral construct into a cell for the first time we suggest using several amounts of shRNA lentiviral particle stock. In addition, we recommend to include one well with cells transduced with Control shRNA Lentiviral Particles (sc-108080).

DAY 3

- Remove the culture medium and replace with 1 ml of complete medium (without Polybrene).
- Incubate the cells overnight.

DAY 4

- To select stable clones expressing the shRNA, split cells 1:3 to 1:5, depending on the cell type, and continue incubating for 24-48 hours in complete medium.

DAY 5-6 and forward

- Select stable clones expressing the shRNA via Puromycin dihydrochloride (sc-108071) selection.
- For puromycin selection, use an amount sufficient to kill the non-transduced cells. Puromycin concentrations ranging from 2 to 10 µg/ml are usually sufficient, but a puromycin tritation is recommended when using a new cell line.
- Replace medium with fresh puromycin-containing medium every 3-4 days, until resistant colonies can be identified. Pick several colonies, expand them and assay them for stable shRNA expression.

NOTE: Resulting puromycin-resistant clones may have varying levels of shRNA expression due to the random integration of the lentiviral construct into the genome of the cell.

NOTE: For shRNA expression analysis by Western Blot, prepare cell lysate as follows:

- Wash cells once with PBS.
- Lyse cells in 100 µl of a 1:1 mixture of 2x Electrophoresis Sample Buffer (sc-24945) and RIPA Lysis Buffer (sc-24948) by gently rocking the 12-well plate or by pipetting up and down.
- Sonicate the lysate on ice if necessary.

NOTE: For shRNA expression analysis by RT-PCR, isolate RNA using the method described by P. Chomczynski and N. Sacchi (1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156-159) or a commercially available RNA isolation kit.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

shRNA LENTIVIRAL PARTICLES SUPPORT REAGENTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
Control shRNA Lentiviral Particles	sc-108080	Control shRNA Lentiviral Particles is available as an alternate negative scrambled shRNA sequence control.	200 µl
copGFP Control Lentiviral Particles	sc-108084	copGFP Control Lentiviral Particles are provided as transduction-ready viral particles.	10-20 transductions
Electrophoresis Sample Buffer	sc-24945	Ready-to-use reducing electrophoresis sample buffer solution for the preparation of protein samples to be separated by SDS-PAGE.	25 ml; 2X concentrate
RIPA Lysis Buffer	sc-24948	For use in mammalian cell lysis, with protease inhibitors. Available in four vials: 1X Lysis buffer, PMSF, protease inhibitor cocktail and sodium orthovanadate.	50 ml
Puromycin dihydrochloride	sc-108071	Available for selection and maintenance of cells transfected with the puromycin-N-acetyl-transferase (pac) gene.	25 mg
Polybrene®	sc-134220	Highly efficient infection reagent used to introduce retroviral vectors into mammalian cells.	1 ml

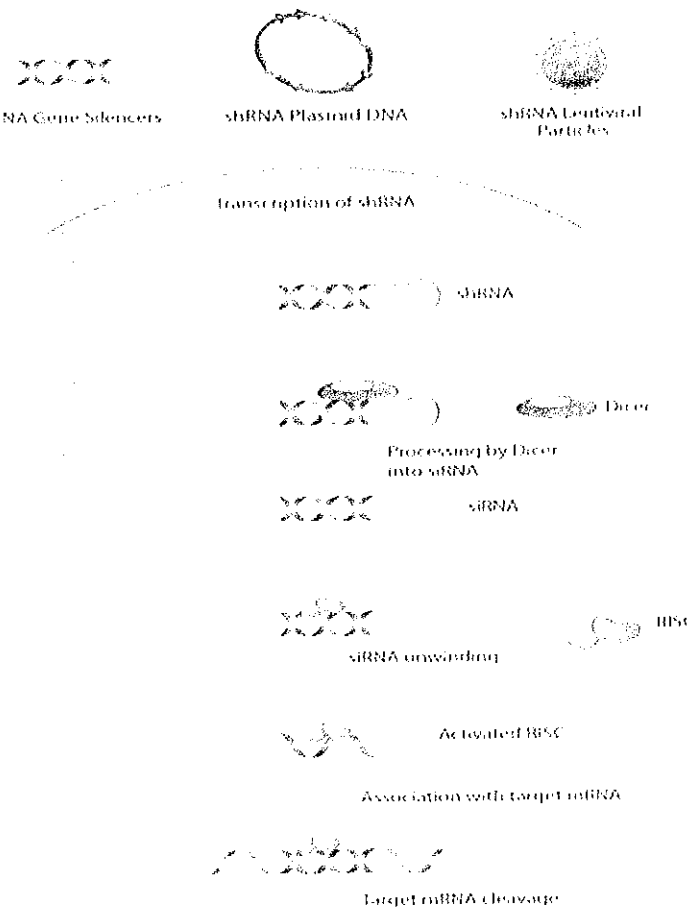
RNAi Gene Silencers

RNA interference (RNAi) was first identified in C. elegans by Nobel laureates Fire and Mello (1), and now represents one of the most promising discoveries in molecular biology. Endogenous RNAi activity has been linked to the regulation of transposon mobility (2), the determination of gene expression profiles (3) and cell fate (4), and is a crucial component of the innate cellular defense against viral infection in vivo(5). Three unique RNAi mechanisms controlling target gene expression have been demonstrated. RNAi regulates gene transcription by modifying heterochromatin formation (6). RNAi exercises two forms of post-transcriptional control. First RNAi can inhibit the translation of target mRNA (7) and second, RNAi can direct target mRNA destruction through the RISC complex (8). DICER first processes dsRNA leaving a two nucleotide long 3' overhang. This primes the dsRNA for binding to the RISC complex and leads to the activation of the enzyme activity of argonaute, the RNAse component of the RISC complex that destroys one of the RNA strands. The remaining guide strand, through complementary binding, then leads the RISC complex to associate with and cleave target RNA molecules.

The discovery of RNAi introduced an extraordinarily powerful laboratory tool for researchers and became a promising potential therapeutic tool, consequently leading to the 2006 Nobel Prize in Physiology or Medicine being awarded to Andrew Z. Fire and Craig C. Mello. In the laboratory, RNAi molecules are being used to downregulate individual target gene expression in a variety of organisms and cell types, exploiting each of the three mechanisms of inhibiting gene expression described above. These techniques are useful for manipulating an experimental system to explore individual gene and protein functions as well as their relationships to other genes and proteins. RNAi also has exciting clinical potential (9).

Details of these RNAi mechanisms are popular subjects of rigorous study, though much remains to be clarified. RNAi control of target mRNA degradation through the RISC complex, however, is the most well-described as well as the intended mechanism for RNAi Gene Silencers.

RNAi-directed mRNA Cleavage



Santa Cruz Biotechnology, Inc. offers a complete line of RNAi Gene Silencers, including siRNA, shRNA Plasmid and shRNA Lentiviral products covering > 99% of human and mouse protein encoding genes.

Click to jump to the section of your choice:

siRNA Gene Silencers shRNA Plasmids shRNA Lentiviral Particles Frequently Asked Questions Back to top

RNA interference Products offered by Santa Cruz Biotechnology Inc.

siRNA description:

- siRNA refers to small interfering or short interfering RNA
- Requires transfection of cells using a lipid-based transfection reagent
- Useful for a transient knock-down

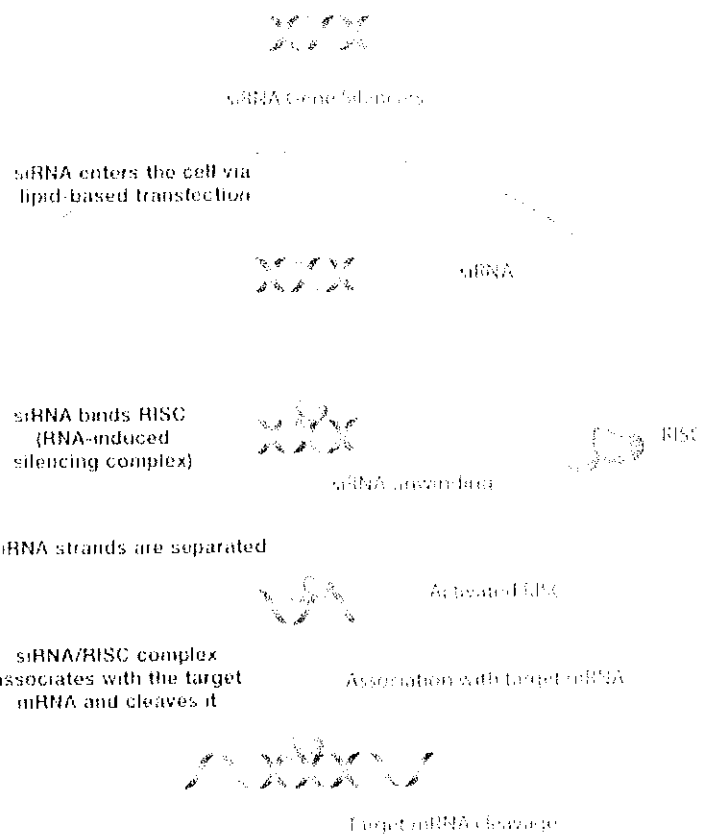
siRNA product details:

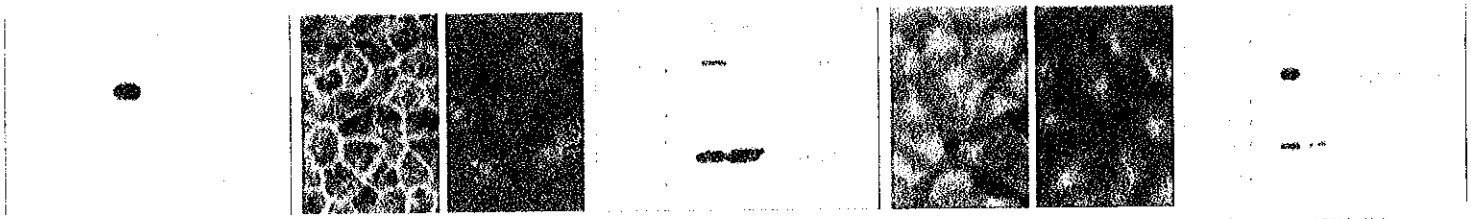
- siRNA Gene Silencers are pools of three target specific 19-25 nucleotide-long double stranded RNA molecules with 2-nt 3' overhangs on each end
- 10 µM, 50-100 transfections
- for independent verification of target gene silencing results, individual siRNA duplex components are also available upon request

Support Products for siRNA Gene Silencers:

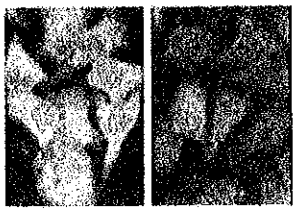
- suitable control antibodies are available
- RT-PCR Primers are available
- siRNA Dilution Buffer, sc-29527
- siRNA Transfection Reagent, sc-29528
- siRNA Transfection Medium, sc-36868
- siRNA Reagent System, sc-45064
- Control siRNAs, including Control siRNA-A, sc-37007
- Control siRNA (FITC Conjugate)-A, sc-36869

How do siRNA Gene Silencers work?





FGF-19 siRNA (h): sc-39480 | CD9 siRNA (h): sc-35032 | Daxx siRNA (h): sc-35178 | Cdc6 siRNA (h): sc-29258 | cytochrome c siRNA (h): sc-29292



cPLA2 siRNA (h): sc-29280 ERK 1 siRNA (m): sc-29308 c-Src siRNA (h): sc-29228 p53 siRNA (h): sc-29435 Lamin A/C siRNA (h): sc-35776

Click to jump to the section of your choice:

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shRNA Plasmid description:

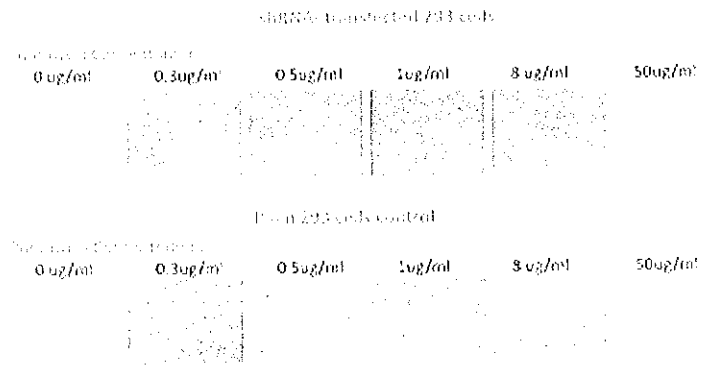
- shRNA refers to small hairpin or short hairpin RNA
- Plasmids encoding shRNA enter the cell via lipid-based transfection
- shRNA plasmids are capable of transient or stable inhibition of target gene expression
- shRNA Plasmids are provided as a pool of three to five lentiviral vector plasmids which each encode a target specific 19-25 nt shRNA with a 6 bp loop
- 20 µg, up to 20 transfections
- shRNA transcription is under the control of the H1 promoter
- provided as transfection-ready purified plasmid DNA
- After transfection, cells stably expressing shRNA can be selected by puromycin treatment

Support Products for shRNA Plasmid Gene Silencers:

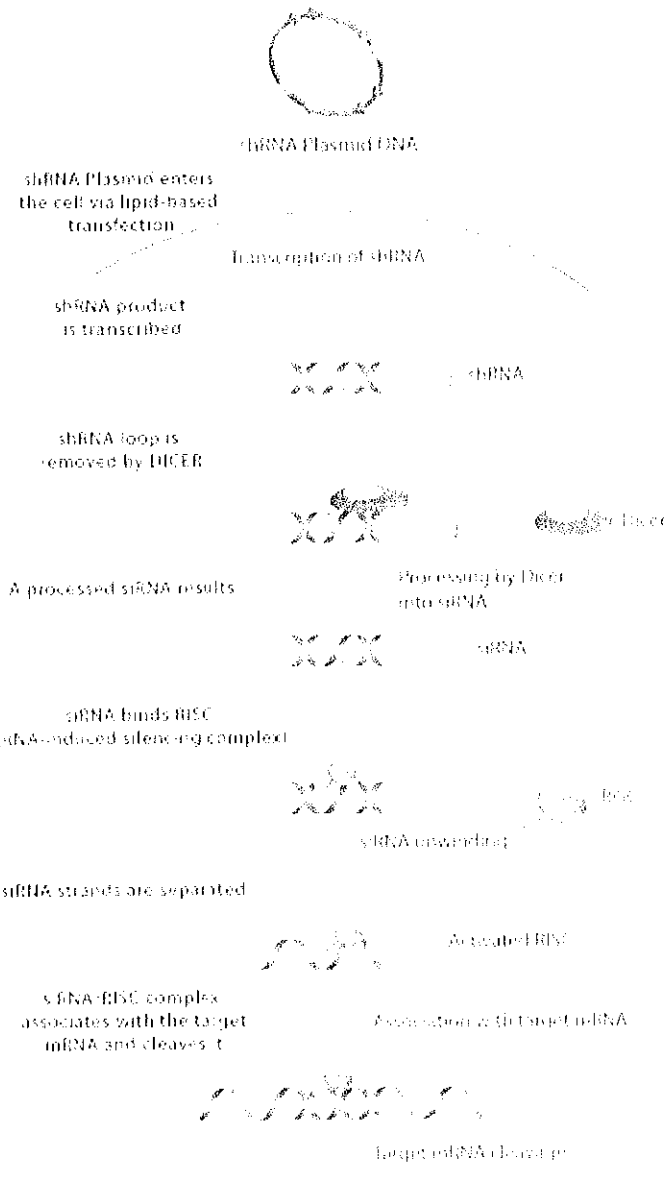
- suitable control antibodies are available
- RT-PCR Primers are available
- shRNA Plasmid Transfection Reagent, sc-108061
- shRNA Plasmid Transfection Medium, sc-108062
- Control shRNA Plasmid-A, sc-108060
- Control shRNA Plasmid-B, sc-108065
- Control shRNA Plasmid-C, sc-108066

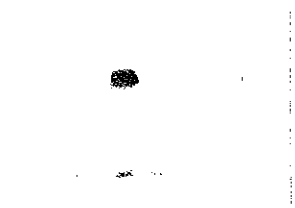
Confirm shRNA Plasmid Gene Silencer transfection efficiency with copGFP Control Plasmid: sc-108083

Generate Cells with stable expression of shRNA

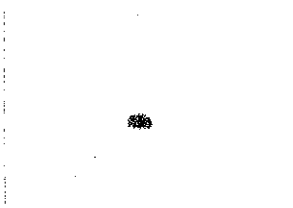


How do shRNA Plasmid Gene Silencers work?



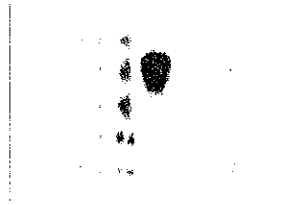


IL-1α shRNA Plasmid (h):
sc-39613-SH

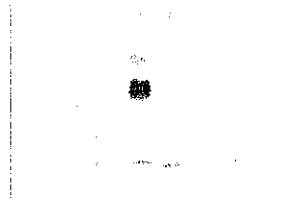


PTN shRNA Plasmid (m):
sc-39714-SH

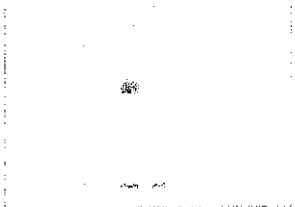
TCF-4 shRNA Plasmid (h):
sc-43525-SH



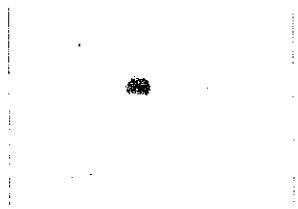
MIS shRNA Plasmid (h):
sc-39793-SH



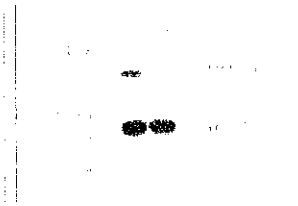
VEGF-D shRNA Plasmid (h):
sc-39844-SH



Amylase shRNA Plasmid (h):
sc-29675-SH



FGF-19 shRNA Plasmid (h):
sc-39480-SH



MMP-9 shRNA Plasmid (h):
sc-29400-SH

BMP-4 shRNA Plasmid (h):
sc-39744-SH

Cyr61 shRNA Plasmid (h):
sc-39331-SH

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How do Lentiviral Particle Gene Silencers work?

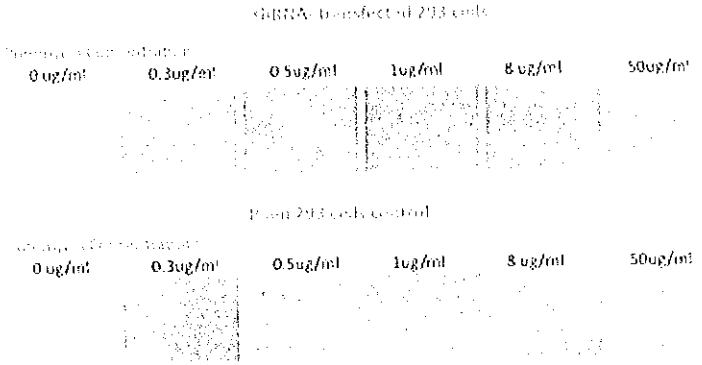
shRNA Lentiviral Particle description:

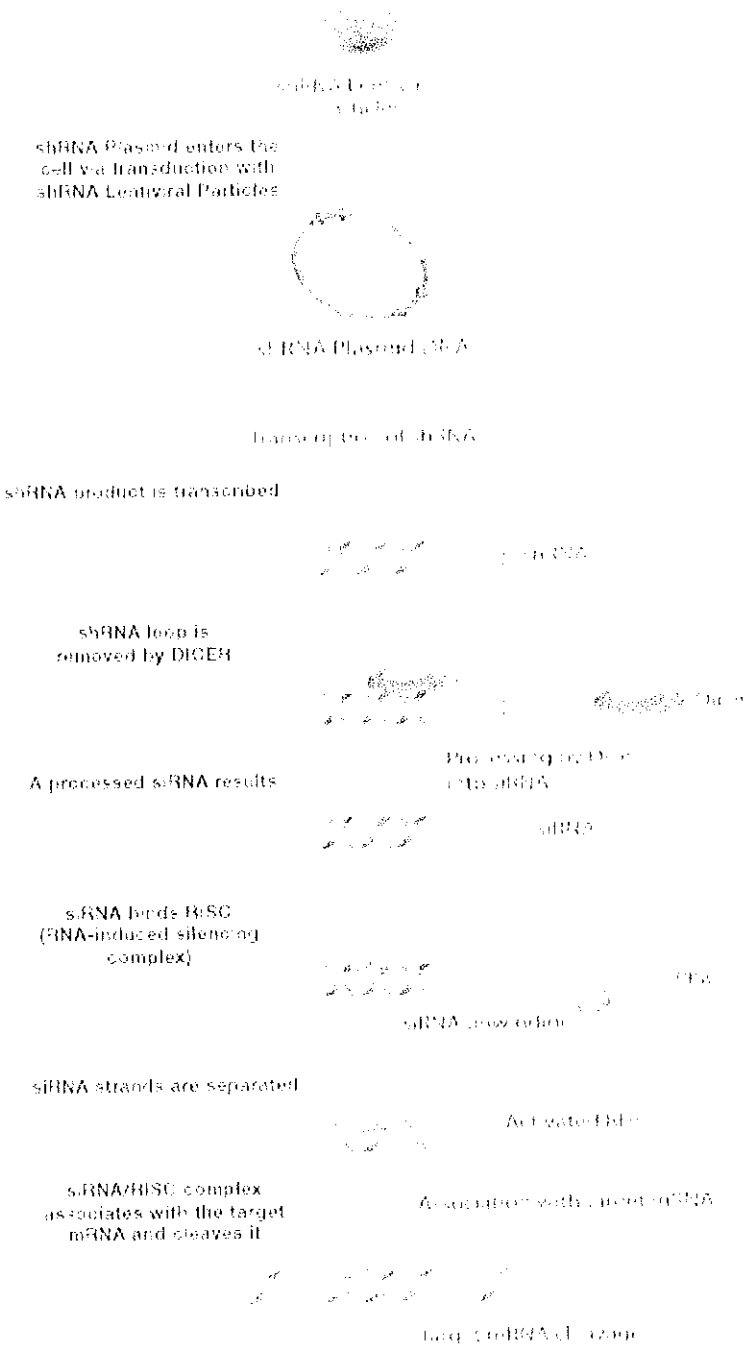
- shRNA refers to small hairpin or short hairpin RNA
- Lentiviral Particles deliver a shRNA encoding plasmid to target cell
- Useful for either transient or stable knock-down of a target gene
- Lentiviral Particles are provided as transduction-ready viruses for targeted gene silencing in mammalian cells (human or mouse)
- 200 µl viral stock containing 10^6 infectious lentiviral transducing particles per ml, sufficient for 10-20 transductions
- The Lentiviral Particles generally contain three to five expression constructs, each construct encoding a target specific 19-25 nt shRNA with a 6 bp loop
- After transduction, cells stably expressing shRNA can be selected by puromycin treatment
- copGFP Control Lentiviral Particles allow confirmation of the transduction efficiency of the Lentiviral Particles in a target cell population by expression of GFP detectable by either flow cytometry or fluorescence microscopy.
- The benefits of using shRNA Lentiviral Particles include avoiding harsh transfection techniques and the ability to introduce shRNA to any cell type
- Biosafety information - Lentiviral Particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into the genomic DNA of target cells.

Support Products for shRNA Lentiviral Particle Gene Silencers:

- suitable control antibodies are available
- RT-PCR Primers are available
- Control shRNA Lentiviral Particles: sc-108080
- copGFP Control Lentiviral Particles: sc-108084
- Puromycin dihydrochloride: sc-108071

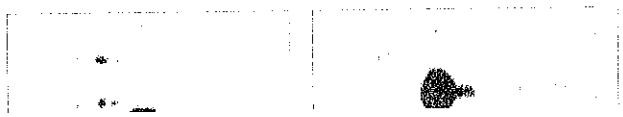
Generate Cells with stable expression of shRNA

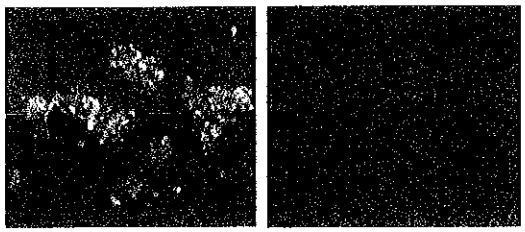




Use an effective Transduction Control

copGFP Control Lentiviral Particles





293T cells stably transduced with copGFP Control Lentiviral Particles (sc-108084) compared with non-transduced 293T cells as a negative control.

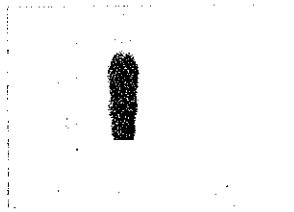
PNP shRNA (h) Lentiviral Particles: sc-45991-V

ephrin-A1 shRNA (m) Lentiviral Particles: sc-39427-V

MCP-4 shRNA Plasmid (h): sc-72122-SH

TNFβ shRNA Plasmid (h): sc-37218-SH

Somatostatin shRNA (h) Lentiviral Particles: sc-39728-V



Fos B shRNA (h) Lentiviral Particles: sc-35403-V

Click to jump to the section of your choice:

siRNA Gene Silencers | shRNA Plasmids | shRNA Lentiviral Particles | Frequently Asked Questions | Back to top

Frequently Asked Questions:

What are the advantages of using shRNA versus siRNA?
Transfection of siRNA Gene Silencers into cultured cells provides a fast and efficient, though short-term, decrease in target gene expression. One may achieve stable gene silencing using shRNA Plasmids or shRNA Lentiviral Particles followed by puromycin selection. So, if one is targeting the expression of a protein with slow turnover, shRNA Plasmid or shRNA Lentiviral Particles would be ideal for accomplishing the goal.

What is the difference between using shRNA Lentiviral Particles versus shRNA Plasmids?
Transfection is required to use shRNA Plasmids for target gene silencing. Whereas shRNA Lentiviral Particles arrive ready to add to virtually any mammalian cell type, including primary and non-dividing cells. Both shRNA Plasmids and shRNA Lentiviral Particles may be used to develop stable expression of the shRNA with puromycin treatment. Lentiviral particles are shipped on dry ice while shRNA Plasmids are shipped on blue ice.

Do Lentiviral shRNA products pose any safety concerns?
Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). The Lentiviral Particles are replication-incompetent and are designed to self-inactivate after transduction and integration of the shRNA constructs into the genomic DNA of target cells.

Are the sequences of your shRNA products the same as those for your related siRNA products to the same gene? Do you make those sequences available?
Yes. The sequences encoded in our shRNA Plasmids are the same as those used in the corresponding siRNA Gene Silencer products. These sequences are available to customers. Contact your Technical Service Representative.

The shRNA Plasmids are provided as a pool of three to five plasmids. Are they provided in separate vials? Are the individual shRNA plasmids of a pooled product sold separately?
The shRNA Plasmid products are provided in one vial. We offer the siRNA strands separately upon request. We may offer the plasmids separately in the future.

What kind of lentiviral vector do you use? What is the "vector name"?
The lentiviral vector we use is a custom made, proprietary vector. Please let us know what information you are looking for and why you need it. We might be able to answer your question without disclosing proprietary information.

What type of promoter does your vector use for shRNA transcription?
The vector uses a H1 promoter

What type of selection marker(s) are in the vector?
The vector has a Puromycin resistance gene encoding puromycin N-acetyltransferase enzyme for selection of successfully transfected or transduced cells.

How do you propagate the lentiviral vector plasmid?
The shRNA Plasmids and Lentiviral Particles are sold as transfection / transduction ready products. No additional preparation is necessary. shRNA Gene Silencers are consumable products for which no propagation protocols are provided.

What is copGFP and how is it helpful for use with the shRNA plasmids and Lentiviral Particles?
By administering the copGFP plasmid or copGFP Lentiviral Particles to a separate sample of target cells, one can identify the transfection or viral transduction efficiency for the target cell population. The copGFP plasmid and copGFP Lentiviral particles lead to expression of copepod green fluorescent protein which can be detected using a fluorescence microscope or flow cytometer.

What is the difference between (h) and (h2) shRNA products (for example E-Cadherin, sc-35242-SH and sc-44222-SH)?
The (h) and (h2) products are designed to silence the same gene, they have different sequences.

What support products and transfection reagents must I purchase from SCBT to use your shRNA Plasmids?
We recommend our shRNA Plasmid DNA Transfection Reagent, sc-108061 in addition to shRNA Plasmid DNA Transfection Medium, sc-108062. We also recommend our control shRNA Plasmid DNAs, either sc-108060 (A), sc-108065 (B) or sc-108066 (C). These encode scrambled shRNA sequences which will not target any known mammalian mRNA.

Click to jump to the section of your choice:

siRNA Gene Silencers shRNA Plasmids shRNA Lentiviral Particles Frequently Asked Questions Back to top

References

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7. Tamura, Y., Yoshida, M., Ohnishi, Y. and Hohjoh, H. 2008. Variation of gene silencing involving endogenous microRNA in mammalian cells. Mol Biol Rep, epub.
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9. Zhanga, Y., Yanga, H., Xiaoa, B., Wub, M., Zhoua, W., Lia, J., Lib, G. and Christados, P. 2008. Dendritic cells transduced with lentiviral-mediated RelB-specific ShRNAs inhibit the development of experimental autoimmune myasthenia gravis. Mol Imunol, epub.

Lentivirus Packaging and Production

The laboratories of Didier Trono (EPFL) and Robert Weinberg (Whitehead institute) have deposited plasmids for the production of lentiviral particles. These plasmids can be used with many lentiviral vectors, including The RNAi Consortium shRNA vectors being distributed by Sigma (i.e. MISSION shRNAs) and Open Biosystems (i.e. TRC shRNAs).

Overview

For producing lentiviral particles, you typically need three components: 1) a lentiviral vector, such as pLKO.1 or pLVTHM, containing the shRNA or transgene, 2) a packaging vector, such as psPAX2 or pCMV-dR8.2 dvpr, and 3) an envelope vector, such as pMD2.G or pCMV-VSVG.

For most applications, you can produce viral particles by transient transfection of 293T cells with a 2nd generation packaging system (e.g. packaging plasmid psPAX2 and envelope plasmid pMD2.G).

2nd Generation Packaging System

In general, lentiviral vectors with a wildtype 5' LTR need the 2nd generation packaging system because these vectors require TAT for activation. All lentiviral vectors from the Trono or Aebischer lab require packaging with a 2nd generation system.

Below are two 2nd generation systems. Lentiviral plasmids based on pLKO.1 can be packaged with either system, although the first system has been reported to produce higher titer. See Addgene's pLKO.1 Protocol for producing lentiviral particles.

2nd generation system deposited by the Trono lab:

ID	Plasmid	Description
12260	psPAX2	2nd generation packaging plasmid for producing viral particles. psPAX2 contains a robust CAG promoter for efficient expression of packaging proteins. Trono lab and Aebischer lab lentiviral vectors require psPAX2. Produces higher titer than pCMV-dR8.2 dvpr.
12259	pMD2.G	Envelope plasmid for producing viral particles

2nd generation system deposited by the Weinberg lab:

ID	Plasmid	Description
8455	pCMV-dR8.2 dvpr	2nd generation packaging plasmid for producing viral particles
8454	pCMV-VSVG	Envelope plasmid for producing viral particles

3rd Generation Packaging System

The 3rd generation packaging system offers maximal biosafety but is more cumbersome to use, as it involves the transfection of four different plasmids in the producer cells (two packaging plasmids, an envelope plasmid, and the lentiviral vector).

If you wish to use this system, you need to have a lentiviral vector with a chimeric 5' LTR in which the HIV promoter is replaced with CMV or RSV, thus making it TAT-independent. Examples of these vectors include pLKO.1, pLL3.7, pLB, p_enti6, pSico/pSicoR, pCL, pCS, and pLove.

Most Aebischer and Trono Lab lentiviral vectors CANNOT be used with this system. A lentiviral vector carrying a chimeric 5' LTR can be packaged with either the 2nd or 3rd generation packaging system.

ID	Plasmid	Description
12251	pMDLg/pRRE	3rd generation packaging plasmid for producing viral particles

12253	pRSV-Rev	3rd generation packaging plasmid for producing viral particles
12259	pMD2.G	Envelope plasmid for producing viral particles

More information

- Click here to browse other RNAi vectors, or search for plasmids using the search bar at the top of the page.
- Trono Lab website or Lentiweb: information and a discussion forum on cloning, packaging, and other protocols.
- Moffat J et. al. 2006. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell 124:1283-1298. (PubMed)
- Ventura et. al. 2004. Cre-lox-regulated conditional RNA interference from transgenes. PNAS 2004 Jul 13;101(28):10380-5. (PubMed)
- Naldini L et. al. 1996. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272:263-267. (PubMed)
- Dull et al., A Third-Generation Lentivirus Vector with a Conditional Packaging System. J. Virol. 1998 72(11): 8463-8472. (PubMed)
- Zufferey R et. al. 1997. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat Biotechnol 15(9):871-5. (PubMed)
- Zufferey R et. al. 1998. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol 72(12):9873-80. (PubMed)

Cell Line

The 293T cell line for producing lentiviral particles can be obtained from GenHunter.

addgene

Search for Plasmids: Go

Advanced Search

Login | New User

Plasmid Cart

Your cart is empty

Recently Viewed

pBABE-neo Plasmid 1767

Browse > Bob Weinberg > Article > pBABE-neo

Print Friendly Email

Price: $65.00

Plasmid 1767: pBABE-neo

	none
	Unknown
	pBABE-neo
(Search Vector Database)	Mammalian expression,Retroviral
	5330
pBABE 5' (List of Sequencing Primers)	
	pBABE 3'
	Ampicillin
	High Copy
	Yes
	Neomycin
View sequence	
View map	
	DH5a
	Bob Weinberg
pBABE protocol (PDF)	
MTA	

Author's map
Sequence
Reviews (0)

From this article
Bob Weinberg Lab Plasmids

pBABE-hygro
pBABE-puro
pBABE-zeo (pBABE-bleo)
pBABE GFP
pBABE-neo-hTERT

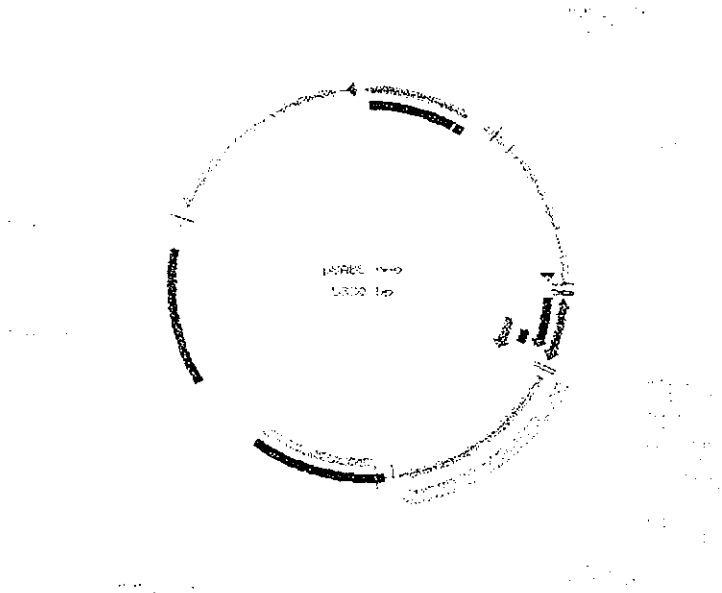
Morgenstern JP, Land H., 1990, Nucleic Acids Research 18(12):3587-96.

Note: There is an extra ~300 bp of vector sequence between the HindIII site and the neomycin gene that is not depicted in the author's sequence.

If you are using the pBABE protocol from the Weinberg Lab to generate virus, please note that Addgene supplies pCL-Eco (#12371), VSV-G (#8454), and a gag/pol expression vector (#8455).

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge



5_LTR2	12 - 479	SpeI	616
3MoMuLV_LTR	18 - 479	AatII	701
psi_plus_pack	549 - 1350	BamHI	1355
pBABE_5_primer	1318 - 1334	EcoRI	1379
pBABE_3_primer	1428 - 1408	SalI	1397
SV40_enhancer	1629 - 1414	StuI	1726
SV40_promoter	1426 - 1694	HindIII	1743
SV40_origin	1593 - 1670	ClaI	2567
SV40_promoter	1546 - 1748	NheI	2641
SV40pro_F_primer	1655 - 1674	NotI	4294
ORF frame 3	1752 - 2564		
NeoR/KanR	1773 - 2561		
3MoMuLV_LTR	2611 - 3204		
5_LTR2	2653 - 3204		
pBR322_origin	4180 - 3561		
Ampicillin	5202 - 4342		
AmpR_promoter	5272 - 5244		

Please acknowledge the principal investigator if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 1767" in your Materials and Methods section.
This information allows Addgene to create a link from the plasmid page to your publication.

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.1
Revision Date 06/18/2009
Print Date 02/03/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : ADP-ribosyltransferase C3, from *Clostridium botulinum*

Product Number : A8724
Brand : Sigma

Company : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA
Telephone : +19058299500
Fax : +19058299292
Emergency Phone # : 800-424-9300

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Botulinum neurotoxin C3
C3 Exotoxin
C3 Transferase
C3 Exoenzyme

CAS-No.	EC-No.	Index-No.	Concentration
ADP-ribosyltransferase C3 from Clostridium botulinum			
58319-92-9	-	-	-

3. HAZARDS IDENTIFICATION

WHMIS Classification
D2B Toxic Material Causing Other Toxic Effects Moderate respiratory irritant

HMIS Classification
Health Hazard: 2
Flammability: 0
Physical hazards: 0

Potential Health Effects

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

4. FIRST AID MEASURES

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

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

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

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

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

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

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

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

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation.

Environmental precautions
Do not let product enter drains.

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

7. HANDLING AND STORAGE

Handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Safety glasses with side-shields conforming to EN166

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Materials to avoid
Strong oxidizing agents

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

no data available

Irritation and corrosion

no data available

Sensitisation

Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals.

Chronic exposure

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

Signs and Symptoms of Exposure

Headache, Dizziness, To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Potential Health Effects

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

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product

Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

15. REGULATORY INFORMATION

DSL Status

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

	CAS-No.
ADP-ribosyltransferase C3 from Clostridium botulinum	58319-92-9

WHMIS Classification

D2B Toxic Material Causing Other Toxic Effects Moderate respiratory irritant

16. OTHER INFORMATION

Further information

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

Phalloidin, *Amanita phalloides*: sc-202763

The Power to Question

1. Identification of the substance/preparation and of the company/undertaking

Product name : **Phalloidin, *Amanita phalloides***

Chemical formula : $C_{35}H_{48}N_8O_{11}S$

Synonym : phalloidin

Catalog # : sc-202763

Supplier : Santa Cruz Biotechnology, Inc.
2145 Delaware Avenue
Santa Cruz, California 95060
800.457.3801 or 831.457.3800

2. Composition / information on ingredients

Substance/ Preparation : Substance

Chemical name	CAS No.	EC Number	Symbol	R-Phrases
Phalloidin *Amanita phalloides*	17466-45-4	241-484-5	T+	R27/28

3. Hazards identification

Physical chemical hazards : Not applicable

Human health hazards : DANGER!
MAY BE FATAL IF ABSORBED THROUGH SKIN OR IF SWALLOWED
MAY CAUSE DAMAGE TO THE FOLLOWING ORGANS: KIDNEYS, LIVER, GASTROINTESTINAL TRACT, CENTRAL NERVOUS SYSTEM.

4. First-aid measures

First-Aid measures

Inhalation : If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Ingestion : If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Skin Contact : In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Eye Contact : Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Most important symptoms

Ingestion : Extremely hazardous in case of ingestion. May be fatal if swallowed.

Skin Contact : Extremely hazardous in case of skin contact (permeator). Severe over-exposure can result in death.

Aggravating conditions : Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

5. Fire-fighting measures

Flammability of the Product : May be combustible at high temperature.

Extinguishing Media

Suitable : SMALL FIRE: Use DRY chemical powder.
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Hazardous thermal decomposition products : These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2, ...), sulfur oxides (SO2, SO3, ...).

Special fire-fighting procedures : Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.

Protection of fire-fighters : Be sure to use an approved/certified respirator or equivalent.

6. Accidental release measures

Personal precautions	:	Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self-contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Small Spill and Leak	:	Use appropriate tools to put the spilled solid in a convenient waste disposal container.
Large Spill and Leak	:	Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Eliminate all ignition sources. Call for assistance on disposal.

7. Handling and storage

Handling	:	Keep locked up. Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not ingest. Do not breathe dust. Avoid contact with skin. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label.
Storage	:	Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 4°C (39.2°F).
Packaging Materials		
Recommended	:	Use original container.

8. Exposure controls/personal protection

Engineering measures	:	Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.
Hygiene measures	:	Wash hands, forearms, and face thoroughly after handling compounds and before eating, smoking, using lavatory, and at the end of day.

Ingredient Name	Occupational Exposure Limits
Phalloidin, *Amanita phalloides*	Not available.

Personal protective equipment

Respiratory system	:	Dust respirator. Be sure to use an approved/certified respirator or equivalent.
Skin and body	:	Lab coat.
Hands	:	Gloves.
Eyes	:	Safety glasses
Protective Clothing - Pictograms	:	

9. Physical and chemical properties

Physical state	:	Solid.
Color	:	Colorless
Molecular Weight	:	788.9 g/mole
Melting Point	:	280 to 282°C (536 to 539.6°F)
Solubility	:	Easily soluble in methanol
Flash point	:	Not available
Explosive properties	:	Risks of explosion of the product in presence of mechanical impact: Not available Risks of explosion of the product in presence of static discharge: Not available

10. Stability and reactivity

Stability	:	The product is stable.
Conditions to avoid	:	Not available
Hazardous Decomposition Products	:	These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2, ...), sulfur oxides (SO2, SO3, ...)

11. Toxicological information

RTECS	:	SE9800000
Local effects		
Skin irritation	:	Not available.
Acute toxicity	:	LD50: Not available. LC50: Not available.
Chronic toxicity	:	Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.
Other Toxic Effects on Humans	:	Toxic for humans or animal life. Extremely hazardous in case of skin contact (permeation) of ingestion.
		Not available.
		Not available
Teratogenic effects	:	Not available.
Mutagenic effects	:	Not available
Reproduction toxicity	:	Not available.
Teratogenic effects	:	Not available.

12. Ecological information

Ecotoxicity	:	Not available
Toxicity of the products of biodegradation	:	The products of degradation are less toxic than the product itself

13. Disposal considerations

Methods of disposal: Waste of residues/ Contaminated packaging	:	Waste must be disposed of in accordance with federal, state and local environmental control regulations

14. Transport information

In accordance with transport regulations		
UN number	:	UN2811
Land - Road/Railway		
Proper shipping name	:	Toxic solid, organic, n.o.s.*
ADR/RID Class	:	CLASS 6.1 Toxic substance.
Packing Group	:	II
ADR/RID label	:	
Sea		
Proper shipping name	:	Toxic solid, organic, n.o.s.*
IMDG Class	:	CLASS 6.1 Toxic substance
Packing Group	:	II
IMDG label	:	
Air		
Proper shipping name		Toxic solid, organic, n.o.s.*
IATA Number	:	UN2811
IATA/ICAO Class	:	CLASS 6.1 Toxic substance.

Packing Group : I

UN Number :

Special Provisions for Transport : Not available

15. Regulatory information

Regulations

Hazard symbols :

Classification : Very toxic

Risk Phrases : R27/28- Very toxic in contact with skin and if swallowed.

Safety Phrases : S22- Do not breathe dust.
S36/37/39- Wear suitable protective clothing, gloves and eye/face protection
S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Contains : - Phalloidin, *Amanita phalloides*

U.S. Federal Regulations : TSCA. No products were found.
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
SARA 313 toxic chemical notification and release reporting: No products were found
Clean Water Act (CWA) 307: No products were found.
Clean Water Act (CWA) 311: No products were found.
Clean air act (CAA) 112 accidental release prevention: No products were found.
Clean air act (CAA) 112 regulated flammable substances: No products were found
Clean air act (CAA) 112 regulated toxic substances: No products were found.

DSCL classification : CLASS: Highly toxic.
CLASS: Target organ effects

State Regulations :

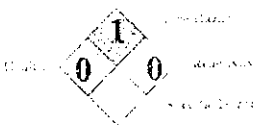
WHMIS (Canada) : Not controlled under WHMIS (Canada)
No products were found

16. Other information

Hazardous Material Information System (U.S.A.)

[illegible]	* 0
[illegible]	1
Reactivity	0
Personal Protection	E

National Fire Protection Association (U.S.A.)



The above information is believed to be correct but does not purport to be complete and should be used only as a guide. The burden of safe use of this material rests entirely with the user.

Emergency Contact:

Santa Cruz Biotechnology, Inc.
2145 Delaware Avenue
Santa Cruz, California 95060
800.457.3801 or 831.457.3800
or Luis Yanez 831.251.2179

ATCC

MATERIAL SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: G418 Sulfate
ATCC Catalog No.: 30-2305

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108
FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710

CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

HAZARDOUS INGREDIENTS	CAS NUMBER	EC NUMBER (EINECS)	
G418	108321-42-2	UNLISTED	

SECTION 3 - HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:
HARMFUL BY INHALATION AND IF SWALLOWED.
AVOID CONTACT WITH SKIN AND EYES.
DO NOT BREATHE DUST.

WHEN USING, DO NOT EAT, DRINK, OR SMOKE.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING AND GLOVES.
IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW LABEL WHERE POSSIBLE).

TARGET ORGAN:
KIDNEYS.
EARS.
EYES.

SECTION 4 - FIRST AID MEASURES

EYES: FLUSH WITH PLENTY OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN.
SKIN: IMMEDIATELY WASH SKIN WITH SOAP AND PLENTY OF WATER.
INGESTION: WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.
INHALATION: REMOVE TO FRESH AIR. IF BREATHING BECOMES DIFFICULT, CALL A PHYSICIAN.

SECTION 5 - FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA:
WATER SPRAY, CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.

SPECIAL FIREFIGHTING PROCEDURES:
WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES.

UNUSUAL FIRE AND EXPLOSIONS HAZARDS:
EMITS TOXIC FUMES UNDER FIRE CONDITIONS. SUBSTANCE IS NONCOMBUSTIBLE.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND RUBBER GLOVES.
WEAR DISPOSABLE COVERALLS AND DISCARD THEM AFTER USE.
SWEEP UP CAREFULLY TO AVOID CREATING AIRBORNE DUST.
PLACE IN A SUITABLE CONTAINER, SEAL, LABEL, AND HOLD FOR WASTE DISPOSAL.
VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE

SECTION 7 - HANDLING AND STORAGE

STORE AT REFRIGERATED TEMPERATURES (4 to 8° C). KEEP CONTAINER TIGHTLY CLOSED.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

MECHANICAL EXHAUST REQUIRED.
WEAR APPROPRIATE NIOSH/MSHA-APPROVED RESPIRATOR, CHEMICAL-RESISTANT GLOVES, SAFETY GOGGLES, AND OTHER PROTECTIVE CLOTHING.
EMERGENCY SHOWER AND EYE WASH STATION SHOULD BE READILY AVAILABLE.
AVOID CONTACT WITH EYES, SKIN AND CLOTHING.
AVOID PROLONGED OR REPEATED EXPOSURE.
WASH THOROUGHLY AFTER HANDLING.
WASH CONTAMINATED CLOTHING BEFORE REUSE.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AND ODOR:

WHITE TO OFF-WHITE ODORLESS POWDER.

PHYSICAL PROPERTIES:
DATA NOT AVAILABLE.

SECTION 10 - STABILITY AND REACTIVITY

STABILITY:
STABLE.

INCOMPATIBILITIES:
STRONG OXIDIZING AGENTS.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS:
CARBON MONOXIDE, CARBON DIOXIDE, NITROGEN OXIDES, SULFUR OXIDES.

HAZARDOUS POLYMERIZATION:
WILL NOT OCCUR.

SECTION 11 - TOXICOLOGICAL INFORMATION

ACUTE EFFECTS:
MAY CAUSE SKIN IRRITATION.
MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN.
MAY CAUSE EYE IRRITATION.
MAY BE HARMFUL IF INHALED.
MATERIAL MAY BE IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT.
MAY BE HARMFUL IF SWALLOWED.
TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.

SECTION 12 - ECOLOGICAL INFORMATION

DATA NOT AVAILABLE.

SECTION 13 - DISPOSAL CONSIDERATIONS

CONTACT A LICENSED WASTE DISPOSAL SERVICE TO DISPOSE OF THIS MATERIAL.
OBSERVE ALL FEDERAL, STATE, AND LOCAL ENVIRONMENTAL REGULATIONS.

SECTION 14 - TRANSPORT INFORMATION

DATA NOT AVAILABLE.

SCHEDULE B NUMBER: 2941.90.6000

SECTION 15 - REGULATORY INFORMATION

EUROPEAN INFORMATION:
RISK PHRASES 20/22
HARMFUL BY INHALATION AND IF SWALLOWED.
SAFETY PHRASES 20/21, 22, 24/25, 26, 36/37, 45
WHEN USING, DO NOT EAT, DRINK, OR SMOKE.
DO NOT BREATHE DUST.
AVOID CONTACT WITH SKIN AND EYES.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING AND GLOVES.
IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY (SHOW LABEL WHERE POSSIBLE).

SECTION 16 - OTHER INFORMATION

July 2003

ATCC

MATERIAL SAFETY DATA SHEET

SECTION 1. CHEMICAL IDENTIFICATION

Product Name: Dimethylsulfoxide (DMSO)
ATCC Catalog No.: 4-X

SECTION 2. COMPOSITION/INFORMATION ON INGREDIENTS

HAZARDOUS INGREDIENTS	CAS NUMBER	EC NUMBER (EINECS)	PERCENTAGE
DIMETHYLSULFOXIDE	67-68-5	200-664-3	99 – 100%

SECTION 3. HAZARDS IDENTIFICATION

LABEL PRECAUTIONARY STATEMENTS:
IRRITANT.
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
COMBUSTIBLE LIQUID.
READILY ABSORBED THROUGH SKIN.

TARGET ORGAN(S):
EYES.
SKIN.
DO NOT BREATHE VAPOR.
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING.
MOISTURE SENSITIVE.

SECTION 4. FIRST-AID MEASURES

IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER.
IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.

SECTION 5. FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA:
WATER SPRAY, CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.

SPECIAL FIREFIGHTING PROCEDURES:
WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES.

UNUSUAL FIRE AND EXPLOSIONS HAZARDS:
EMITS TOXIC FUMES UNDER FIRE CONDITIONS.
COMBUSTIBLE LIQUID.
METHYL SULFOXIDE (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 METHYL SULFOXIDE DEGRADES GIVING OFF FORMALDEHYDE, METHYL MERCAPTAN, AND SULFUR DIOXIDE.

SECTION 6. ACCIDENTAL RELEASE MEASURES

WEAR RESPIRATOR, CHEMICAL SAFETY GOGGLES, RUBBER BOOTS AND HEAVY RUBBER GLOVES.
ABSORB ON SAND OR VERMICULITE AND PLACE IN CLOSED CONTAINERS FOR DISPOSAL.
VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE. EVACUATE AREA.
AVOID CONTAMINATING WATER SUPPLY.

SECTION 7. HANDLING AND STORAGE

REFER TO SECTION 8.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

MECHANICAL EXHAUST REQUIRED.
SAFETY SHOWER AND EYE BATH.
WASH THOROUGHLY AFTER HANDLING.
KEEP TIGHTLY CLOSED.
KEEP AWAY FROM HEAT AND OPEN FLAME.
STORE IN A COOL DRY PLACE AT 2-8°C.
NIOSH/MSHA-APPROVED RESPIRATOR.
COMPATIBLE CHEMICAL-RESISTANT GLOVES.
CHEMICAL SAFETY GOGGLES.
DO NOT BREATHE VAPOR.
AVOID CONTACT WITH DMSO SOLUTIONS CONTAINING TOXIC MATERIALS OR MATERIALS WITH UNKNOWN TOXICOLOGICAL PROPERTIES. DIMETHYL SULFOXIDE IS READILY ABSORBED THROUGH SKIN AND MAY CARRY SUCH MATERIALS INTO THE BODY.
AVOID PROLONGED OR REPEATED EXPOSURE.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AND ODOR:
CLEAR, COLORLESS LIQUID.
HYGROSCOPIC.
GARLIC-LIKE ODOR.

BOILING POINT:	189°C
MELTING POINT:	18.4°C
FLASHPOINT:	188.60°F/87°C
EXPLOSION LIMITS IN AIR:	
UPPER	28.5 %
LOWER	2.6 %
VAPOR PRESSURE:	0.42 MMHG @ 20°C
SOLUBILITY:	
WATER -Z1076	
ALCOHOLS, ETHYL	
SPECIFIC GRAVITY:	1.1 G
VAPOR DENSITY:	2.7 G/L
FREEZING POINT:	18.5°C

SECTION 10. STABILITY AND REACTIVITY

STABILITY:
STABLE.

INCOMPATIBILITIES:
PROTECT FROM MOISTURE.
ACID CHLORIDES.
PHOSPHORUS HALIDES.
STRONG ACIDS.
STRONG OXIDIZING AGENTS.
STRONG REDUCING AGENTS.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS:
CARBON MONOXIDE.
CARBON DIOXIDE.
SULFUR OXIDES.
FORMALDEHYDE.
MERCAPTANS.

HAZARDOUS POLYMERIZATION:
WILL NOT OCCUR.

SECTION 11. TOXICOLOGICAL INFORMATION

ACUTE EFFECTS:
TO THE BEST OF OUR KNOWLEDGE, THE CHEMICAL, PHYSICAL, AND TOXICOLOGICAL PROPERTIES HAVE NOT BEEN THOROUGHLY INVESTIGATED.
CAUSES SKIN IRRITATION.
READILY ABSORBED THROUGH SKIN.
MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN.
CAUSES EYE IRRITATION.
MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT

MAY BE HARMFUL IF INHALED.
MAY BE HARMFUL IF SWALLOWED.
LD.
LC.
AVOID CONTACT WITH DMSO SOLUTIONS CONTAINING TOXIC MATERIALS OR MATERIALS WITH UNKNOWN TOXICOLOGICAL PROPERTIES. DIMETHYL SULFOXIDE IS READILY ABSORBED THROUGH SKIN AND MAY CARRY SUCH MATERIALS INTO THE BODY.

CHRONIC EFFECTS.
TARGET ORGAN(S):
EYES.
SKIN.

RTECS #: PV6210000:
METHYL SULFOXIDE.

IRRITATION DATA:

SKN-RBT 10 MG/24H OPEN MLD	AIHAAP 23,95,1962
SKN-RBT 500 MG/24H MLD	85JCAE -,1044,1986
EYE-RBT 100 MG	TXAPA9 39,129,1977
EYE-RBT 500 MG/24H MLD	85JCAE -,1044,1986

TOXICITY DATA:

ORL-RAT LD50:14500 MG/KG	TXAPA9 15,74,1969
SKN-RAT LD50:40 GM/KG	ANYAA9 141,96,1967
IPR-RAT LD50:8200 MG/KG	FCTOD7 22,665,1984
SCU-RAT LD50:12 GM/KG	ARZNAD 14,1050,1964
IVN-RAT LD50:5360 MG/KG	TXAPA9 7,104,1965
UNR-RAT LD50:1300 MG/KG	NTIS** AD-A159-418
ORL-MUS LD50:7920 MG/KG	CHTPBA 3,10,1968
SKN-MUS LD50:50 GM/KG	ANYAA9 141,96,1967
IPR-MUS LD50:2500 MG/KG	RPTOAN 35,300,1972
SCU-MUS LD50:14 GM/KG	ANYAA9 141,96,1967
IVN-MUS LD50:3100 MG/KG	TXAPA9 15,74,1969
UNR-MUS LD50:12 GM/KG	USXXAM #4767763
ORL-DOG LD50:>10 GM/KG	ANYAA9 141,96,1967
IVN-DOG LD50:2500 MG/KG	CNCRA6 31,7,1963
ORL-CKN LD50:12 GM/KG	JPPMAB 15,688,1963
ORL-MAM LD50:21400 MG/KG	GISAAA 39(4),86,1974
ORL-BWD LD50:100 MG/KG	TXAPA9 21,315,1972

TARGET ORGAN DATA:
BEHAVIORAL (ALTERED SLEEP TIME).
LUNGS, THORAX OR RESPIRATION (DYSPNAE).
LUNGS, THORAX OR RESPIRATION (CYANOSIS).
GASTROINTESTINAL (NAUSEA OR VOMITING).
LIVER (JAUNDICE, OTHER OR UNCLASSIFIED).
BLOOD (OTHER CHANGES)

EFFECTS ON FERTILITY (PRE-IMPLANTATION MORTALITY).
EFFECTS ON EMBRYO OR FETUS (FETOTOXICITY).
SPECIFIC DEVELOPMENTAL ABNORMALITIES (MUSCULOSKELETAL SYSTEM).
ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION.

SECTION 12. ECOLOGICAL INFORMATION

DATA NOT YET AVAILABLE.

SECTION 13. DISPOSAL CONSIDERATIONS

CONTACT A LICENSED PROFESSIONAL WASTE DISPOSAL SERVICE TO DISPOSE OF THIS MATERIAL.
THIS COMBUSTIBLE MATERIAL MAY BE BURNED IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER.
OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.

SECTION 14. TRANSPORT INFORMATION

THIS PRODUCT CONTAINS NO HAZARDOUS SUBSTANCES AS DEFINED BY THE DEPARTMENT OF TRANSPORTATION REGULATIONS, CODIFIED IN TITLE 49 CFR SECTION 171.8 AT REPORTABLE QUANTITIES ACCORDING TO TABLE 1 OF APPENDIX A OF 49 CFR §172.101.

SECTION 15. REGULATORY INFORMATION

EUROPEAN INFORMATION:
IRRITANT.
R 36/37/38
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
S 23
DO NOT BREATHE VAPOR.
S 26
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
S 36
WEAR SUITABLE PROTECTIVE CLOTHING.

REVIEWS, STANDARDS, AND REGULATIONS:
OEL=MAK.
OEL-GERMANY: NO MAK ESTABLISHED, JAN1999.
OEL-RUSSIA: STEL 20 MG/M3, JAN1993.
OEL-SWEDEN: TWA 50 PPM (150 MG/M3), KTV 150 PPM (500 MG/M3), SKIN, JAN1999.
OEL-SWITZERLAND: MAK-W 50 PPM (160 MG/M3), SKIN, JAN1999.
NOHS 1974: HZD 80564; NIS 11; TNF 476; NOS 25; TNE 22461.
NOES 1983: HZD 80564; NIS 29; TNF 3507; NOS 40; TNE 44947; TFE 16837.
EPA GENETOX PROGRAM 1988, POSITIVE: ASPERGILLUS-ANEUPLOIDY; S CEREVISIAE GENE CONVERSION.

EPA GENETOX PROGRAM 1988. NEGATIVE: SHE-CLONAL ASSAY: CELL TRANSFORM.-MOUSE EMBRYO.
EPA GENETOX PROGRAM 1988. NEGATIVE: CELL TRANSFORM.-RLV F344 RAT EMBRYO.
EPA GENETOX PROGRAM 1988. NEGATIVE: D MELANOGASTER-WHOLE SEX CHROM LOSS: HOST-MEDIATED ASSAY.
EPA GENETOX PROGRAM 1988. NEGATIVE: N CRASSA-ANEUPLOIDY: E COLI POLA WITH S9.
EPA GENETOX PROGRAM 1988. NEGATIVE: HISTIDINE REVERSION-AMES TEST: IN VITRO SCE-NONHUMAN.
EPA GENETOX PROGRAM 1988. NEGATIVE: D MELANOGASTER SEX-LINKED LETHAL.
EPA GENETOX PROGRAM 1988. INCONCLUSIVE: ASPERGILLUS-RECOMBINATION: CARCINOGENICITY-MOUSE/RAT.
EPA GENETOX PROGRAM 1988. INCONCLUSIVE: D MELANOGASTER-RECIPROCAL TRANSLOCATION.
EPA GENETOX PROGRAM 1988. INCONCLUSIVE: RODENT DOMINANT LETHAL: B SUBTILIS REC ASSAY.
EPA GENETOX PROGRAM 1988. INCONCLUSIVE: E COLI POLA WITHOUT S9 EPA TSCA SECTION 8(B) CHEMICAL INVENTORY.
EPA TSCA SECTION 8(D) UNPUBLISHED HEALTH/SAFETY STUDIES EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE. JANUARY 2001.

SECTION 16. OTHER INFORMATION

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

March 2002