THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 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: Dr. Stephen Ferguson
SIGNATURE:
DEPARTMENT: Molecular Brain Research Group
ADDRESS: Rm 3250, Robarts
PHONE NUMBER: 21165
EMERGENCY PHONE NUMBER(S):
EMAIL: ferguson@robarts.ca

Location of experimental work to be carried out: Building(s) Robarts Room(s) 3250

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, HSFO
GRANT TITLE(S): Dynamic Regulation of mGluR signalling
Regulation of CREI/5HT2 Receptor Signalling Complexes
Regulation of GPCR signalling, desensitization + resensitization

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:

Lianne Dale
Ana Magalhaes
Jessica Esseltine
Henry Dyn
Christie Godin
Fabiola Ribeiro
Tamara Cregan
maryse Paquet
Sandra Fakim

* 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 ○ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)?
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ○ YES ☒ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	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 NM522/XL-1 Blue	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500ml	Invitrogen Stratagene	☒ 1 ○ 2 ○ 3
ADENOVIRUS	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	100 mL		○ 1 ☒ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 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 ○ NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	○ Yes ☒ No		Not applicable
Rodent	☒ Yes ○ No	Mouse	2005-084-11
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ No		

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

▷Level 2 cell lines (all others are Level 1)
- HEK293
- COS7

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	HEK293, IMR32, U87	ATCC
Rodent	☒ Yes ○ No	A10, RBL-2H3, PC12	"
Non-human primate	☒ Yes ○ No	COS7	"
Other (specify)	○ Yes ☒ No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		○ Yes ○ No		○1 ○2 ○3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○1 ○2 ○3
Human Organs or Tissues (unpreserved)		○ Yes ○ No		○1 ○2 ○3
Human Organs or Tissues (preserved)		○ Yes ○ No		○1 ○2 ○3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α E.Coli XL1 Blue	See attached list	See attached list	Too many to list	

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Adenovirus	Adeno MR, GPR30, GFP, SH MR	Microbix Biosystem	G Protein Coupled Receptor Kinase (GRK)	Overexpress GRK

* Please attach a Material Safety Data Sheet or equivalent.

* Virus is supplied by we do not amplify

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ☒YES ○ NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used mouse

6.3 AUS protocol # 2005-084-11

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

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

8.0 Biological Toxins

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

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

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

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

8.5 How much of the toxin is stored*? _____

*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 ☒ 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:

_____
_____
_____

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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.

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SIGNATURE _____

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 ☒ 2 O 3

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

☒ YES, permit # if on-campus 2006-10
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: Sept 10/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: Ronald Worsley
Date: September 15, 2009

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

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

Special Conditions of Approval:

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

The Ferguson Lab studies the signaling and regulation of G protein coupled receptors (GPCRs).

All of our studies use cultured cells from either established cell lines or primary cultures isolated from the mouse. The COS7 and HEK 293 cell lines are biosafety level 2 whereas all of the others are level 1.

All of our studies involve either endogenously expressed proteins or proteins overexpressed by transfection with various cDNAs. In general, our expression plasmids are either commercially available or slightly modified variations of the commercial plasmids. We have several hundred cDNAs that code for various wild-type, dominant negative or constitutively active GPCRs or other proteins involved in the GPCR signaling and/or receptor regulation.

The various cDNA constructs are purified from cultures of DH5a and XL1 Blue E.Coli that have been transformed with the various expression constructs.

The adenovirus is used to introduce the GRK into the mouse primary neuronal cultures since primary cultures are difficult to transfect.

None of these agents will be used in live animals.

Note: The adenovirus is supplied by the Gros Lab / Feldman Lab

LD

Ron Noseworthy

From: Lianne B. Dale [ldale4@uwo.ca]
Sent: September 14, 2009 4:36 PM
To: Ron Noseworthy
Subject: Re: RE: Adenovirus MSDS

Hi Ron,

Fabiola says it is second generation.

Lianne

----- Original Message -----
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Monday, September 14, 2009 4:30 pm
Subject: RE: Adenovirus MSDS
To: ldale@robarts.ca

> Hi Lianne,
>
> Can you let me know if the virus is a first or second generation?
>
> Thanks
>
> Ron
>

> **From:** Lianne B. Dale [mailto:ldale4@uwo.ca]
> **Sent:** September 14, 2009 3:44 PM
> **To:** Ron Noseworthy
> **Subject:** Adenovirus MSDS
>
> Hi Ron,
>
> I asked Fabiola about the MSDS for the adenovirus. She gets the virus from Qingming in Gros Lab. He told her he looked for one but couldn't find it.
>
> Lianne
>
> Robarts Research Institute
> 100 Perth Drive
> London, ON, Canada
> N6A 5K8
>
> Tel: (519) 663-5777 x24165
> Fax: (519) 663-3314
>
>

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

only one available
no up date

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045
http://www.vectorbiolabs.com

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

3701 Market Street, Suite 340, Philadelphia, PA 19104 Tel: 877-biolabs Fax: 215-966-6001 info@vectorbiolabs.com

invitrogen

Material Safety Data Sheet

Revision Date: 09-Nov-2006

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

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

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

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

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	5-10

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

3. HAZARDS IDENTIFICATION

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

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

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Glycerol	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

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

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

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

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

End of Safety Data Sheet

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

Material Safety Data Sheet

Agilent Technologies

Stratagene XL1-Blue Subcloning-Grade Competent Cells, Catalog # 200130

1. Product and company identification

Product name	:	**Stratagene XL1-Blue Subcloning-Grade Competent Cells, Catalog # 200130**
Part No.	:	XL1-Blue Subcloning-Grade Competent Cells 200130-41 pUC18 Control Plasmid DNA 200231-42
Manufacturer / Supplier	:	Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Emergency telephone number	:	1-800-894-1304
Use of the substance/preparation	:	Analytical chemistry.
Validation date	:	11/21/2008

2. Hazards identification

Physical state	:	XL1-Blue Subcloning-Grade Competent Cells	Liquid.
		pUC18 Control Plasmid DNA	Liquid.
Odor	:	XL1-Blue Subcloning-Grade Competent Cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
OSHA/HCS status	:	XL1-Blue Subcloning-Grade Competent Cells pUC18 Control Plasmid DNA	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
Emergency overview-Signal Word	:	WARNING !	
Emergency overview-Label Statement	:	XL1-Blue Subcloning-Grade Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
		pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
		XL1-Blue Subcloning-Grade Competent Cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
Routes of entry	:	XL1-Blue Subcloning-Grade Competent Cells	Eye contact. Inhalation. Ingestion.
		pUC18 Control Plasmid DNA	Eye contact. Ingestion.

<u>Potential acute health effects</u>

2 . Hazards identification

Eyes	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Skin	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Inhalation	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Ingestion	:	XL1-Blue Subcloning-Grade Competent Cells	Toxic if swallowed.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Medical conditions aggravated by over-exposure	:	XL1-Blue Subcloning-Grade Competent Cells	Repeated or prolonged exposure to the substance can produce target organs damage.
		pUC18 Control Plasmid DNA	Not applicable.
Over-exposure signs/symptoms	:	XL1-Blue Subcloning-Grade Competent Cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
XL1-Blue Subcloning-Grade Competent Cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5

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

4 . First aid measures

Eye contact	:	XL1-Blue Subcloning-Grade Competent Cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	XL1-Blue Subcloning-Grade Competent Cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are

4 . First aid measures

			severe.
Inhalation	:	XL1-Blue Subcloning-Grade Competent Cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
Ingestion	:	XL1-Blue Subcloning-Grade Competent Cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	XL1-Blue Subcloning-Grade Competent Cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
Notes to physician	:	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5 . Fire-fighting measures

Flammability of the product	:	XL1-Blue Subcloning-Grade Competent Cells	Non-flammable.
		pUC18 Control Plasmid DNA	Non-flammable.
Products of combustion	:	XL1-Blue Subcloning-Grade Competent Cells	Decomposition products may include the following materials: carbon oxides halogenated compounds metal oxide/oxides
		pUC18 Control Plasmid DNA	No specific data.
Extinguishing media			
Suitable	:	XL1-Blue Subcloning-Grade Competent Cells	Use an extinguishing agent suitable for the surrounding fire.
		pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	XL1-Blue Subcloning-Grade Competent Cells	Not applicable.
		pUC18 Control Plasmid DNA	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	XL1-Blue Subcloning-Grade Competent Cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

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

7 . Handling and storage

Handling	:	XL1-Blue Subcloning-Grade Competent Cells	Do not ingest. Wash thoroughly after handling.
		pUC18 Control Plasmid DNA	Wash thoroughly after handling.
Storage	:	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.	

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
XL1-Blue Subcloning-Grade Competent Cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust
Manganese dichloride	**ACGIH TLV (United States, 1/2008).**

8 . Exposure controls/personal protection

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

Consult local authorities for acceptable exposure limits.

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

Personal protection

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

Skin : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Other protection : Not available.

Hygiene measures : Handle as biohazard material (Biosafety level 1).
Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	: XL1-Blue Subcloning-Grade Competent Cells	Liquid.
	pUC18 Control Plasmid DNA	Liquid.
Flash point	: XL1-Blue Subcloning-Grade Competent Cells	Not applicable.
	pUC18 Control Plasmid DNA	Not applicable.

9 . Physical and chemical properties

Color	:	XL1-Blue Subcloning-Grade Competent Cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
Odor	:	XL1-Blue Subcloning-Grade Competent Cells	Not available.
		pUC18 Control Plasmid DNA	Not available.
pH	:	XL1-Blue Subcloning-Grade Competent Cells	Neutral.
		pUC18 Control Plasmid DNA	Neutral.
Boiling/condensation point	:	XL1-Blue Subcloning-Grade Competent Cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 116.29°C (241.3°F)
		pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
Melting/freezing point	:	XL1-Blue Subcloning-Grade Competent Cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 1.7°C (35.1°F)
		pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
Relative density	:	XL1-Blue Subcloning-Grade Competent Cells	Weighted average: 1.41 (Water = 1)
		pUC18 Control Plasmid DNA	Not available.
Vapor pressure	:	XL1-Blue Subcloning-Grade Competent Cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
Vapor density	:	XL1-Blue Subcloning-Grade Competent Cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.83 (Air = 1)
		pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).

10 . Stability and reactivity

Stability and reactivity	:	The product is stable.
Incompatibility with various substances	:	Reactive or incompatible with the following materials: oxidizing materials and acids.
Hazardous decomposition products	: XL1-Blue Subcloning-Grade Competent Cells	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
	pUC18 Control Plasmid DNA	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

11 . Toxicological information

Acute toxicity

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

Eyes	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.

11 . Toxicological information

Skin	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Inhalation	:	XL1-Blue Subcloning-Grade Competent Cells	No known significant effects or critical hazards.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Ingestion	:	XL1-Blue Subcloning-Grade Competent Cells	Toxic if swallowed.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards.

Classification

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

Potential chronic health effects

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

Over-exposure signs/symptoms

Inhalation	:	No specific data.
Ingestion	:	No specific data.
Skin	:	No specific data.
Eyes	:	No specific data.

Other adverse effects	:	XL1-Blue Subcloning-Grade Competent Cells	Not available.
		pUC18 Control Plasmid DNA	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

Aquatic ecotoxicity

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

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

13 . Disposal considerations

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

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

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

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

14 . Transport information

Regulatory information

DOT /IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification	: XL1-Blue Subcloning-Grade Competent Cells	Toxic material Target organ effects
	pUC18 Control Plasmid DNA	Not regulated.
U.S. Federal regulations	: XL1-Blue Subcloning-Grade Competent Cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
	XL1-Blue Subcloning-Grade Competent Cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard
	pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
	XL1-Blue Subcloning-Grade Competent Cells	**Clean Water Act (CWA) 307**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.

15 . Regulatory information

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

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

16 . Other information

Label requirements	: XL1-Blue Subcloning-Grade Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
	pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

Date of issue : 11/21/2008

Version : 1

Notice to reader

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

☑ **Indicates information that has changed from previously issued version.**

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 
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:	

Related Links ▸

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

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

Comments:

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006

Cell Biology

ATCC® Number:	**CCL-127™** Order this Item	Price:	**$264.00**

Designations:	IMR-32
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast; neuroblast 
Source:	**Organ:** brain **Disease:** neuroblastoma **Cell Type:** neuroblast;
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:** April, 1967
Applications:	transfection host (technology from amaxa)
Virus Susceptibility:	vesicular stomatitis (Indiana); herpes simplex; vaccinia; coxsackievirus B3; poliovirus 3 (poorly)
Virus Resistance:	echovirus 11
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9 D16S539: 8 D5S818: 11,12 D7S820: 9,10 THO1: 7,9.3 TPOX: 11 vWA: 15
Cytogenetic Analysis:	Stable male karyotype with stemline number of 49. Two large marker chromosomes with submedian centromeres. A deletion in one number 1 chromosome: One number 16 chromosome missing; two extra chromosomes in C group. Sublines with 50 and 48 chromosomes differ from those with 49 chromosomes by having an extra or missing C group chromosome respectively.
Isoenzymes:	G6PD, B

Related Links ▸

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

Age:	13 months
Gender:	male
Ethnicity:	Caucasian
Comments:	The IMR-32 cell line was established by W.W. Nichols, J. Lee and S. Dwight in April, 1967 from an abdominal mass occurring in a 13-month-old Caucasian male. [22190] The tumor was diagnosed as a neuroblastoma with rare areas of organoid differentiation. Two cell types are present. Predominant is a small neuroblast-like cell. The other is a large hyaline fibroblast. The cell line was submitted to the American Type Culture Collection in the 36th passage. It has been demonstrated that the cells can be propagated successfully beyond the 80th serial subculture.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM 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.Maintain cultures at a cell concentration between 4 X 10 exp4 and 4 X 10 exp5 cells/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Doubling Time:	approximately 20 hrs.
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22190: Tumilowicz JJ, et al. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res. 30: 2110-2118, 1970. PubMed: 5459762 32287: Rostomily RC, et al. Expression of neurogenic basic helix-loop-helix genes in primitive neuroectodermal tumors. Cancer Res. 57: 3526-3531, 1997. PubMed: 9270024 32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

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Cell Biology

ATCC® Number:	**HTB-14™** Order this Item	Price:	**$256.00**

Designations:	U-87 MG
Depositors:	J Ponten
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** brain **Tumor Stage:** classified as grade IV as of 2007 **Disease:** glioblastoma; astrocytoma
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)
Tumorigenic:	Yes
Antigen Expression:	Blood Type A, Rh+
Cytogenetic Analysis:	This is a hypodiploid human cell line with the modal chromosome number of 44 occurring in 48% of cells. The rate of higher ploidy was 5.9%., Twelve markers were common to all cells, including der(1)t(1;3) (p22;q21), der(16)t(1;16) (p22;p12), del(9) (p13) and nine others. The marker der(1) had two copies in most cells. There was only one copy of normal X. N1, N6 and N9 were not found.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 1 PGM1, 2 PGM3, 1
Age:	44 years
Gender:	female
Ethnicity:	Caucasian

Related Links ▸

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

Comments:	This is one of a number of cell lines derived from malignant gliomas (see also ATCC HTB-15 and ATCC HTB-16) by J. Ponten and associates from 1966 to 1969. Mycoplasma contamination was eliminated in September 1975.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** 5% CO2 in air recommended **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:5 is recommended **Medium Renewal:** 2 to 3 times 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 medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22159: Beckman G, et al. G-6-PD and PGM phenotypes of 16 continuous human tumor cell lines. Evidence against cross-contamination and contamination by HeLa cells. Hum. Hered. 21: 238-241, 1971. PubMed: 4332744 22536: Fogh J, et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. J. Natl. Cancer Inst. 58: 209-214, 1977. PubMed: 833871 22539: Fogh J, et al. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. J. Natl. Cancer Inst. 59: 221-226, 1977. PubMed: 327080 23094: Olopade OI, et al. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res. 52: 2523-2529, 1992. PubMed: 1568221 23128: Ponten J, Macintyre EH. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. 74: 465-486, 1968. PubMed: 4313504 32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: 8855306

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Cell Biology

ATCC® Number:	**CRL-1476™**	Order this Item	Price:	**$268.00**

Designations:	A-10
Depositors:	W Carlisle
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	myoblast 
Source:	**Strain:** DB1X **Organ:** aorta, thoracic **Tissue:** medial layer
Cellular Products:	myokinase; creatine phosphokinase; myosin
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)
Age:	embryo
Comments:	The clonal cell line A10 was derived by B. Kimes and B. Brandt from the thoracic aorta of DB1X embryonic rat and possesses many of the properties characteristic of smooth muscle cells. The cells produce spontaneous action potentials at the stationary phase of the growth cycle and exhibit an increase in activity of the enzymes myokinase and creatine phosphokinase.
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

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Every 3 to 4 days

Preservation:
Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 29 hours

Related Products:
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:
1061: Kimes BW, Brandt BL. Characterization of two putative smooth muscle cell lines from rat thoracic aorta. Exp. Cell Res. 98: 349-366, 1976. PubMed: 943301
32281: Zhang X, et al. Microfilament depletion and circumvention of multiple drug resistance by sphinxolides. Cancer Res. 57: 3751-3758, 1997. PubMed: 9288783
32468: Gordon EM, et al. Factor XII-induced mitogenesis is mediated via a distinct signal transduction pathway that activates a mitogen-activated protein kinase. Proc. Natl. Acad. Sci. USA 93: 2174-2179, 1996. PubMed: 8700904
32530: Zhang X, Smith CD. Microtubule effects of welwistatin, a cyanobacterial indolinone that circumvents multiple drug resistance. Mol. Pharmacol. 49: 288-294, 1996. PubMed: 8632761

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Cell Biology

ATCC® Number:	**CRL-2256™**	Order this Item	Price:	**$268.00**

Designations:	RBL-2H3
Depositors:	RP Siraganian
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	fibroblast
Source:	**Organ:** peripheral blood **Strain:** Wistar **Disease:** basophilic leukemia **Cell Type:** basophil; chemically induced
Cellular Products:	histamine
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
Receptors:	FcERI (Fc of IgE)

Related Links ▸

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

Comments:

RBL-2H3 is a basophilic leukemia cell line isolated and cloned in 1978 in the Laboratory of Immunology at the National Institute of Dental Research from Wistar rat basophilic cells that were maintained as tumors. [22638]
These cells have high affinity IgE receptors.
They can be activated to secrete histamine and other mediators by aggregation of these receptors or with calcium ionophores.
They have been used extensively to study FcERI and the biochemical pathways for secretion in mast cells.
RBL-2H3 cells have been the model for studies of structure of FcERI.
They have been used extensively for studies of different aspects of secretion in cells including the role of changes in intracellular calcium, the activation of phospholipases, protein kinases and small G proteins.
Although nearly all lots of fetal bovine serum support the growth of these cells, the cells grown in some lots degranulate better after FcERI aggregation.
Another rat basophil line is available (RBL-1, see ATCC CRL-1378) that does not degranulate.
Histamine release capacity may be seriously reduced after too much subculturing. PubMed: 6166481.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: heat-inactivated fetal bovine serum to a final concentration of 15%.
Temperature: 37.0°C

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: *A subcultivation ratio of 1:4 to 1:8 is recommended*
Medium Renewal: *Every 2 to 3 days*

Preservation:
Freeze medium: Complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor temperature

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

References:
1232: Kulczycki A Jr., et al. The interaction of IgE with rat basophilic leukemia cells. I. Evidence for specific binding of IgE. J. Exp. Med. 139: 600-616, 1974. PubMed: 4812630
22475: Barsumian EL, et al. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. Eur. J. Immunol. 11: 317-323, 1981. PubMed: 6166481
22638: Eccleston E, et al. Basophilic leukaemia in the albino rat and a demonstration of the basopoietin. Nat. New Biol. 244: 73-76, 1973.

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Cell Biology

ATCC® Number: **CRL-1721™** Order this Item Price: **$256.00**

Additional information about this cell line

Designations:	PC-12
Depositors:	B Patterson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	loosely adherent, multicell aggregates
Organism:	Rattus norvegicus (rat)
Morphology:	polygonal 
Source:	**Organ:** adrenal gland **Disease:** pheochromocytoma
Cellular Products:	catecholamines; dopamine; norepinephrine [1163]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
Receptors:	nerve growth factor (NGF), expressed
Tumorigenic:	Yes
Cytogenetic Analysis:	40 chromosomes; 38 autosomes plus XY [1163]
Gender:	male
Comments:	The PC-12 cell line was derived from a transplantable rat pheochromocytoma. [1163] The cells respond reversibly to NGF by induction of the neuronal phenotype. [1163] The cells do not synthesize epinephrine. [1163]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 2.5%; horse serum to a final concentration of 15%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

Related Links ▸

- NCBI Entrez Search
- Cell Micrograph
- Cell Adherence for PC-12
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Subculturing:	**Protocol:** Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.1. Remove and discard old culture medium.2. Pipet 10 ml fresh medium over the cell sheet and scrape.3. Aspirate cells with a small bore pipette to break up clusters.4. Add appropriate aliquots of the cell suspension to new 75 cm2 flask with 15 ml fresh growth medium. Seed flask at 1.0 x 10(4) to 3.0x 10(4) viable cells / cm2.Or use subcultivation ratio of 1:3 twice weekly Subculture when cell density reaches between 1.0x 1 0(5) to 2.0x 10(5) viable cells / cm2.5. Place culture vessels in incubator at 37°C.PC-12 cells adhere poorly to plastic and tend to grow in small patches of loosely attached cells.Attachment can be enhanced by coating the flasks with Bovine Collagen I or using Corning® CellBIND® Surface Flasks (Free Samples) **Subcultivation Ratio:** 1:3 twice weekly **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020 recommended serum:ATCC 30-2040
References:	1162: Levi A, et al. Molecular cloning of a gene sequence regulated by nerve growth factor. Science 229: 393-395, 1985. PubMed: 3839317 1163: Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA 73: 2424-2428, 1976. PubMed: 1065897 22344: Biocca S, et al. A macromolecular structure favouring microtubule assembly in NGF- differentiated pheochromocytoma cells (PC12). EMBO J. 2: 643-648, 1983. PubMed: 6641712 33014: Weber E, et al. Distinct functional properties of Rab3A and Rab3B in PC12 neuroendocrine cells. J. Biol. Chem. 271: 6963-6971, 1996. PubMed: 8636125

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Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$264.00**

Designations:	COS-7
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast 
Source:	**Organ:** kidney **Cell Type:** SV40 transformed
Cellular Products:	T antigen
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)
Virus Susceptibility:	SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C

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

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70

#	~~cDNA~~ Plasmid	DESCRIPTION	SOURCE
1	pcDNA1-amp		Invitrogen
2	pcDNA3		Invitrogen
3	pcDNA3 HA1		Invitrogen
4	pcDNA3 myc-1		Invitrogen
5	pEGFP-N1		Clontech
6	pEGFP-N2		Clontech
7	pEGFP-N3		Clontech
8	pEGFP-C1		Clontech
9	pEGFP-C2		Clontech
10	pEGFP-C3		Clontech
11	pEGFP w/o ATG	GFP start codon mutated	Clontech
12	pEGFP-1 (delta CMV)		Clontech
13	pEGFP-C2 Link 2		Clontech
14	pEGFP-C2 Link 1		Clontech
15	DsRed1-C1		Clontech
16	DsRed1-C2	pieter	Clontech
17	DsRed1-N1		Clontech
18	DsRed2-N1		Clontech
19	D2Red2-C1		Clontech
20	DsRed2-C3	Alex, made from DsRed2-C1 by adding	Clontech
21	pECFP-C1		Clontech
22	pECFP-N1		Clontech
23	pEYFP-C1		Clontech
24	pEYFP-N1		Clontech
25	pEBG		???derived from pEF-BOS
26	pEBG (3-4)		???derived from pEF-BOS
27	pEBG 2 -5		???derived from pEF-BOS
28	pEBG 4		???derived from pEF-BOS
29	pGEX 4T1		Amersham
30	pGEX 4T Link 1		Amersham
31	pGEX 4T Link 2		Amersham
32	pMAL c2x		New England Biolabs
33	pcDNA2.1 His B		Invitrogen
34	pGAD10		??
35	pGAD10 linkA		??
36	pGAD10 link B		??
37	pAS2-1		ATCC
38	pAS-2-2-15		ATCC
39	pAS2-2-3		ATCC
40	pRluc-N1		PerkinElmer
41	pRluc-N2		PerkinElmer
42	pRluc-N3		PerkinElmer
43	pmRFP		??
44	pmRFP-N1		??
45	PA-GFP-N1	photoactivatable GFP	??
46	PA-GFP-C1	photoactivatable GFP	??
47	pBFP-N1		Clontech

#42 -44

BRET^2 TM

TECHNICAL DATA SHEET

BIOLUMINESCENCE RESONANCE ENERGY TRANSFER

RENILLA LUCIFERASE FUSION PROTEIN EXPRESSION VECTOR

Product: Codon Humanized pRluc-N Vectors

Catalog number: 6310220

Description: **The codon humanized pRluc-N vectors** contain a multiple cloning site (MCS) located upstream of the codon humanized *Renilla* Luciferase gene (Rluc(h)) which acts as the Donor moiety in a BRET^2 assay. The MCS allows for the subcloning of a gene of interest in order to create a fusion protein having the structure [gene of interest:Rluc(h)]. The Rluc codons have been humanized to ensure higher expression levels of the fusion protein in mammalian cells. The fusion protein gene is placed under the control of the cytomegalovirus (CMV) promoter thus assuring a very high constitutive expression in a variety of cells.

Amount: 10 µg lyophilized plasmid DNA (store lyophilized plasmid at –20°C)

Reconstitution Protocol

Reconstitution:
- Centrifuge briefly to recover contents
- Reconstitute to 0.4 µg/µl with 25 µl of 10 mM Tris-HCl pH 8.0, 1 mM EDTA

Storage conditions:
- Store reconstituted plasmid at -20°C
- After thawing, centrifuge briefly to recover contents

Shelf life:
- 1 year from date of receipt under recommended storage conditions

Quality Control Procedures

- The identity of the codon humanized pRluc-N plasmids and the presence of the MCS restriction sites are confirmed by sequence analysis.
- The presence of RNA and chromosomal DNA as well as the proportion of superhelical DNA are determined by agarose gel electrophoresis using 1 µg of plasmid DNA.
- The absence of nuclease contamination is determined by agarose gel electrophoresis following incubation of 1 µg of plasmid DNA in standard restriction buffer for 16 hours at 37°C.
- The quantity and purity of DNA are determined by UV spectroscopy.
- The functionality of the plasmids is assessed by measuring luciferase activity upon transfection of CHO or BHK cells with LipofectAMINE™. The intensity of the luciferase signal is compared to the signal of reference plasmids using a Fusion™ Universal Microplate Analyzer.

Renilla Luciferase Substrate

BRET^2 requires a modified form of the Rluc coelenterazine substrate, called DeepBlueC™. DeepBlueC has been selected for its ability to confer superior spectral properties to the reaction, resulting in excellent discrimination of the Rluc and GFP^2 signals.

BioSignal Packard

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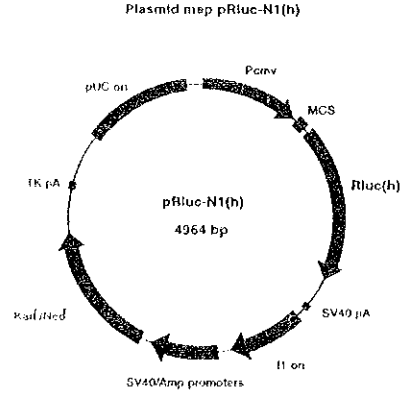
Tel (514) 937-1010 • (800) 293-4501 (US/Canada) • Fax (514) 937-0777 • Email: biosignal@biosignal.com

Codon Humanized pRluc-N1 Vector Map & Notes

Plasmid size: 4964 bp

Cloning sites: BglII, XhoI, MluI, PstI, EcoRV, HindIII, KpnI, SacII, ApaI, SmaI, BamHI

Antibiotic resistance: Prokaryotic: Kanamycin (25 µg/ml for *E.coli*)
Eukaryotic: G418/Neomycin (concentration is cell type dependent)



P_{CMV}	1 - 583
SV40 early poly (A) signal	1783 - 1833
Rluc (h) gene	698 - 1633
TK poly (A) signal	3890 - 3953
Multiple cloning site (MCS)	609 - 680
P_{SV40}/P_{amp}	2397 - 2811
Kan/Neo^R	2860 - 3654
f1 origin	1883 - 2335
pUC sequences (ori)	4239 - 4882

Codon Humanized pRluc-N1 Vector Multiple Cloning Site

Codon humanized pRluc-N1 Vector

AG ATC TGG AGC TCT CGA GAA TTC TCA CGC GTC TGC AGG ATA TCA AGC TTG
BglII XhoI MluI PstI EcoRV HindIII

CGG TAC CGC GGG CCC GGG ATC CA CCG GCT AGA GCC ACC ATG →
KpnI ApaI BamHI* hRluc
SacII SmaI

* Frame changes characterizing humanized pRluc-N1, N2 and N3 vectors occur after this site.

Quality Control Data

- The identity of the codon humanized pRluc-N1 plasmid and the presence of the MCS restriction sites have been confirmed by sequence analysis.
- Incubation in standard restriction enzyme buffer at 37°C for 16 hours showed no evidence of nuclease activity as detected by agarose gel electrophoresis.
- No RNA and chromosomal DNA were detected in a 1 µg sample of plasmid DNA following agarose gel electrophoresis.
- Percent DNA in Superhelical form: > 75%
- Purity (A_{260}/A_{280}) at pH 8.0: 1.76
- Transfection of CHO cells showed that the codon humanized pRluc-N1 vector is functional and expressed Rluc levels within 25% of the corresponding reference plasmids.

6310220
Rev. A 11/00

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invitrogen

Material Safety Data Sheet

Revision Date: 17-Apr-2006

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

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

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

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

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

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

3. HAZARDS IDENTIFICATION

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

Form
Solid

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Sensitization	No information available
Target Organ Effects	No information available

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Solid

Important Health Safety and Environmental Information

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

Water solubility	No data available

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
Not regulated

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

U.S. State Regulations

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

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

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

End of Safety Data Sheet

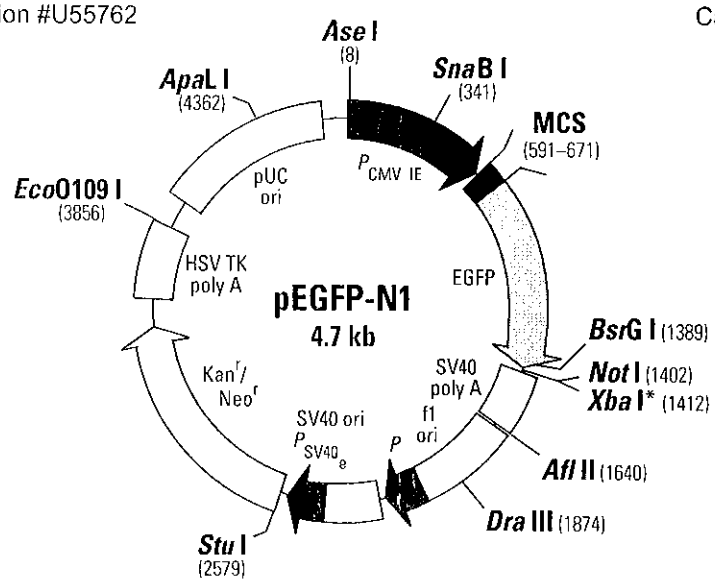
Vector Information

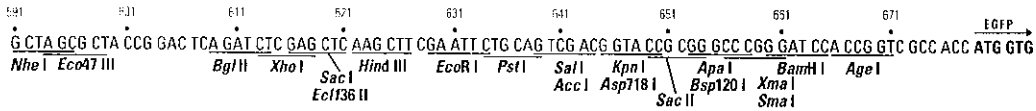
pEGFP-N1 Vector Information

PT3027-5

GenBank Accession #U55762

Catalog #6085-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1 Vector. All restriction sites shown are unique. The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ and make fresh DNA.

Description

pEGFP-N1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo'), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-N1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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E-mail: tech@clontech.com
www.clontech.com

(PR29972; published 03 October 2002)

Use

Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  - Enhancer region:59–465; TATA box: 554–560
  - Transcription start point: 583
  - C→G mutation to remove *Sac* I site: 569
- MCS: 591–671
- Enhanced green fluorescent protein (EGFP) gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1396–1398
  - Insertion of Val at position 2: 682–684
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 871–876
  - His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1552–1557 & 1581–1586; mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104 (Packages the noncoding strand of EGFP.)
- Bacterial promoterfor expression of Kan^r gene:
  - –35 region: 2166–2171; –10 region: 2189–2194
  - Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
  - 21-bp repeats: 2425–2445, 2446–2466 & 2468–2488
  - Early promoter element: 2501–2507
  - Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2629–2631; stop codon: 3421–3423
  - G→A mutation to remove *Pst* I site: 2811
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

Propagation in *E. coli*

- Suitable host strains: DH5a, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

Vector Backbone: pEBG

Vector Type:	Mammalian
Viral/Non-viral:	Non-viral
Promoter:	EF-1alpha
Backbone Size (bp):	6100
Tag:	GST (N terminal)
Bacteria Resistance:	Amp

Comments: Derived from pEF-BOS, BstXI-NotI stuffer fragment of pEF-BOS replaced with polylinker containing BamHI site, PCR to generate GST fragment from pGEX-2T with 5' BglII site and eukaryotic ribosome binding site and 3' BamHI site, inserted into BamHI site to generate pEBG.

More information: Mayer et al. 1995 Current Biology 5(3):296-305.

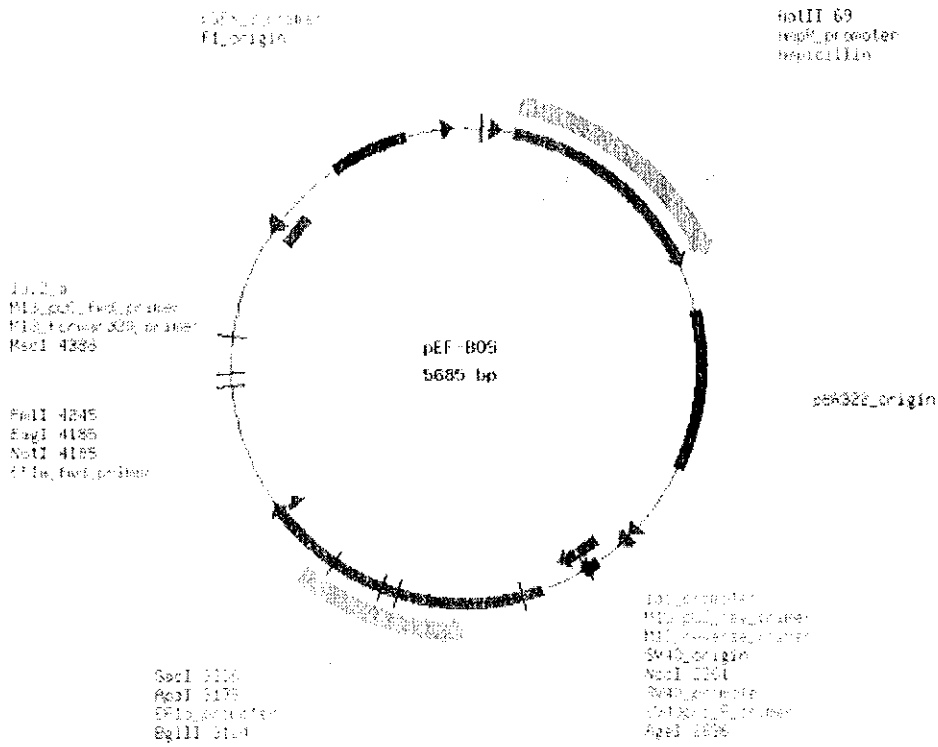
Vector Backbone: pEF-BOS

Vector Type:	Mammalian
Viral/Non-viral:	Non-viral
Backbone Size (bp):	5800
Bacteria Resistance:	Amp
Sequence and Map:	Sequence (Click to see features and cutters)

Comments: SV40 ori (311 bp), human EF-1alpha promoter (1.2 kb), stuffer fragment from CDM8 vector (450 bp, cDNA to be expressed can be inserted by removing the stuffer using BstXI or XbaI), poly-A from human G-CSF (700 bp Eco81I-EcoRI fragment), into HindIII-EcoRI site of pUC119.

More information: Mizushima and Nagata, Nucleic Acids Research, 18(17)5322.

Click on map to enlarge

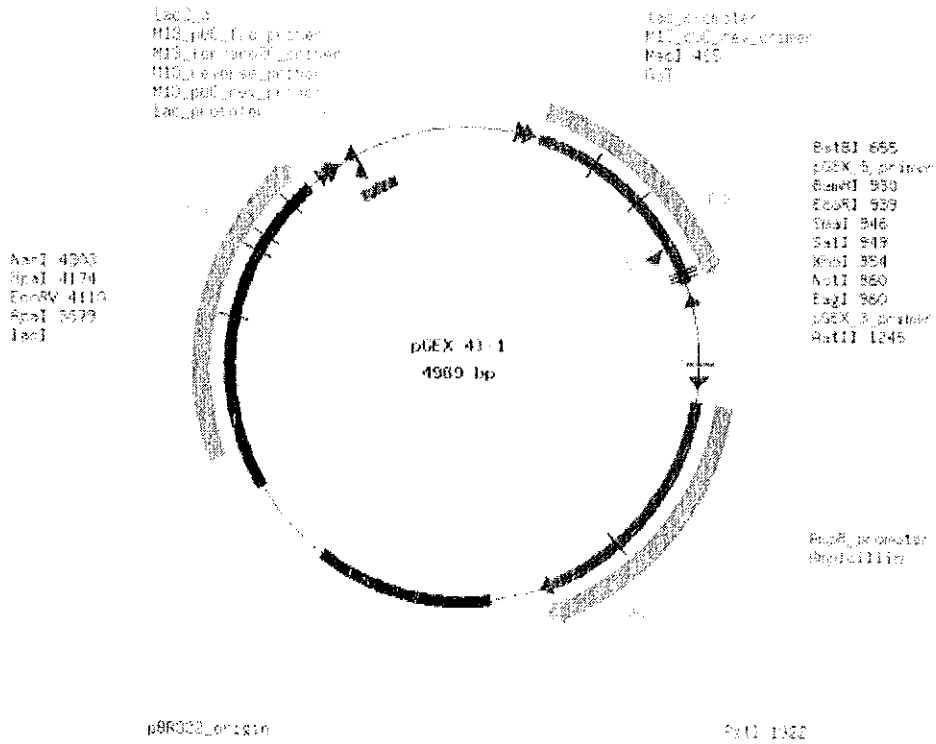


Vector Backbone: pGEX-4T-1

Vendor:	Amersham
Vector Type:	Bacterial
Viral/Non-viral:	Nonviral
Stable/Transient:	Transient
Constitutive/Inducible:	Constitutive
Promoter:	tac
Expression Level:	High (activate with IPTG)
Backbone Size (bp):	4900
Sequencing Primer:	pGEX Fwd
Sequencing Primer Sequence:	5'd[GGGCTGGCAAGCCACGTTTGGTG]3'
Tag:	GST (Nterm)
Bacteria Resistance:	Ampicillin
Mammalian Selection:	N/a
Catalog Number:	27-4580-01
Sequence and Map:	Sequence (Click to see features and cutters)

Comments: Thrombin cleavage site; can directly insert cDNA from lambda gt11 libraries

Click on map to enlarge



GE Healthcare

pGEX Vectors (GST Gene Fusion System)

- **A tac promoter for chemically inducible, high-level expression.**
- An internal lac I^q gene for use in any E. coli host.
- Very mild elution conditions for release of fusion proteins from the affinity matrix, thus minimizing effects on antigenicity and functional activity.
- PreScission™, thrombin, or Factor Xa protease recognition sites for cleaving the desired protein from the fusion product.

Page down for more information

Order Information

Product	Pack size	Product Code	Price	Qty
Glutathione S-transferase Gene Fusion Vectors*				
PGEX-5X-3	1 EA	27-4586-01	CAN$630.00	

* All vectors include E. coli BL21 cells. Additional information about vectors is found at www.gelifesciences.com/pGEX.
* All vectors include E. coli BL21 cells.
All of the GST gene fusion vectors offer:

Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site

pGEX Vectors (GST Gene Fusion System)

Technical Information

Thirteen pGEX vectors are available (see figure). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors. pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3 each encode the recognition sequence for site-specific cleavage by PreScission™ Protease, (see PreScission Protease) between the GST domain and the multiple cloning site. pGEX-4T-1, pGEX-4T-2, and pGEX-4T-3 are derived from pGEX-2T and contain a thrombin protease recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX5X-3 are derivatives of pGEX-3X and possess a factor Xa protease recognition site.

Download the pGEX sequence map in PDF format. For ASCII format please scoll down.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labeling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from heart muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labeled using protein kinase and [γ-^{32}P]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T; its fusion proteins can be cleaved with thrombin.

Cleavage of pGEX-6P GST fusion proteins occurs between the Gln and Gly residues of the recognition sequence Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro [2]. Low temperature (5°C) digestion minimizes the degradation of the protein of interest. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed from the cleavage mixture simultaneously with the GST portion of the fusion protein. The pGEX-6P Expression Vectors permit convenient site-specific cleavage and simultaneous purification on Glutathione Sepharose™. The pGEX-6P series provides all three translational reading frames linked between the GST coding region and the multiple cloning site.

Collectively, the pGEX vectors provide all three translational reading frames beginning with the EcoR I restriction site. pGEX-1λT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from λ gt11 libraries.

Vector	Unformatted	Formatted	GenBank Accession No.
pGEX-1 lambda T, 27-4805-01	*ASCII*	*PDF*	U13849
pGEX-2T, 27-4801-01	*ASCII*	*PDF*	U13850
pGEX-2TK, 27-4587-01	*ASCII*	*PDF*	U13851
pGEX-3X, 27-4803-01	*ASCII*	*PDF*	U13852
pGEX-4T-1, 27-4580-01	*ASCII*	*PDF*	U13853
pGEX-4T-2, 27-4581-01	*ASCII*	*PDF*	*PDF* U13855

pGEX-5X-1, 27-4584-01	ASCII	PDF	U13856
pGEX-5X-2, 27-4585-01	ASCII	PDF	U13857
pGEX-5X-3, 27-4586-01	ASCII	PDF	U13858
pGEX-6P-1, 27-4597-01	ASCII	PDF	U78872
pGEX-6P-2, 27-4598-01	ASCII	PDF	U78873
pGEX-6P-3, 27-4599-01	ASCII	PDF	U78874

Click on "ASCII" to download an unformatted sequence for use by a sequence analysis program. Click on "PDF" to download a formatted sequence and restriction site table. If you prefer accessing the sequence in GenBank, refer to the right-hand column for the GenBank accession number:

- **Expression:** Proteins are expressed as fusion proteins with the 26 kDa glutathione S-transferase (GST). The GST gene contains an ATG and ribosome-binding site, and is under control of the tac promoter. A translation terminator is provided in each reading frame. The resulting fusion protein may be purified using the GST Purification Module (27-4570-01, -02; see GST Purification Modules.)
- **Enzymatic cleavage with PreScission™ Protease:** pGEX-6P-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with PreScission™ Protease. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed simultaneously with the GST portion of the fusion protein.
- **Enzymatic cleavage with thrombin:** pGEX-1 lambda T, pGEX-2T, pGEX-2TK, pGEX-4T-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with thrombin.
- **Enzymatic cleavage with factor Xa:** pGEX-3X, pGEX-5X-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with factor Xa.
- **Direct labeling *in vitro*:** pGEX-2TK allows for direct labeling of fusion proteins in vitro with 32P using the catalytic subunit of cAMP-dependent protein kinase.
- **Host(s):** *E. coli*. The plasmid provides lac Iq repressor.
- **Selectable marker(s):** Plasmid confers resistance to 100 µg/ml ampicillin.
- **Amplification:** Recommended.

Properties of pGEX Vectors • Induction: tac promoter inducible with 1-5 mM IPTG.

• pGEX-1 Lambda T Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935
- MCS: 930-944
- Beta-lactamase gene region: Promoter: -10: 1308-1313; -35: 1285-1290; Start codon (ATG): 1355; Stop codon (TAA): 2213
- *lacIq* gene region: Start codon (GTG): 3296; Stop codon (TGA): 4376
- Plasmid replication region: Site of replication initiation: 2973; Region necessary for replication: 2280-2976
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1019-997

• pGEX-2T Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935
- MCS: 930-945
- Beta-lactamase gene region: Promoter: -10: 1309-1314; -35: 1286-1291; Start codon (ATG): 1356; Stop codon (TAA): 2214
- *lacIq* gene region: Start codon (GTG): 3297; Stop codon (TGA): 4377
- Plasmid replication region: Site of replication initiation: 2974; Region necessary for replication: 2281-2977
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1020-998

• pGEX-2TK Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935;
- Coding for kinase recognition site: 936-950
- MCS: 951-966
- Beta-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
- *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
- Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998

Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-3X Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
- MCS: 934-949
- Beta-lactamase gene region: Promoter: -10: 1313-1318; -35: 1290-1295; Start codon (ATG): 1360; Stop codon (TAA): 2218
- *lacIq* gene region: Start codon (GTG): 3301; Stop codon (TGA): 4381
- Plasmid replication region: Site of replication initiation: 2978; Region necessary for replication: 2285-2981
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1024-1002

• pGEX-4T-1 Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935
- MCS: 930-966
- Beta-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
- *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
- Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-4T-2 Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935
- MCS: 930-967
- Beta-lactamase gene region: Promoter: -10: 1331-1336; -35: 1308-1313; Start codon (ATG): 1378; Stop codon (TAA): 2236
- *lacIq* gene region: Start codon (GTG): 3319; Stop codon (TGA): 4399
- Plasmid replication region: Site of replication initiation: 2996; Region necessary for replication: 2303-2999
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1042-1020

• pGEX-4T-3 Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935
- MCS: 930-965
- Beta-lactamase gene region: Promoter: -10: 1329-1334; -35: 1306-1311; Start codon (ATG): 1376; Stop codon (TAA): 2234
- *lacIq* gene region: Start codon (GTG): 3317; Stop codon (TGA): 4397
- Plasmid replication region: Site of replication initiation: 2994; Region necessary for replication: 2301-2997
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1040-1018

• pGEX-5X-1 Control Regions:
- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for factor Xa cleavage: 921-932
- MCS: 934-969
- Beta-lactamase gene region: Promoter: -10: 1333-1338; -35: 1310-1315; Start codon (ATG): 1380; Stop codon (TAA): 2238
- *lacIq* gene region: Start codon (GTG): 3321; Stop codon (TGA): 4401
- Plasmid replication region: Site of replication initiation: 2998; Region necessary for replication: 2305-3001
- Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1044-1022

- **pGEX-5X-2 Control Regions:**
  - Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for factor Xa cleavage: 921-932
  - MCS: 934-970
  - Beta-lactamase gene region: Promoter: -10: 1334-1339; -35: 1311-1316; Start codon (ATG): 1381; Stop codon (TAA): 2239
  - *lacIq* gene region: Start codon (GTG): 3322; Stop codon (TGA): 4402
  - Plasmid replication region: Site of replication initiation: 2999; Region necessary for replication: 2306-3002
  - Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1045-1023

- **pGEX-5X-3 Control Regions:**
  - Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for factor Xa cleavage: 921-932
  - MCS: 934-971
  - Beta-lactamase gene region: Promoter: -10: 1335-1340; -35: 1312-1317; Start codon (ATG): 1382; Stop codon (TAA): 2240
  - *lacIq* gene region: Start codon (GTG): 3323; Stop codon (TGA): 4403
  - Plasmid replication region: Site of replication initiation: 3000; Region necessary for replication: 2307-3003
  - Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1046-1024

References

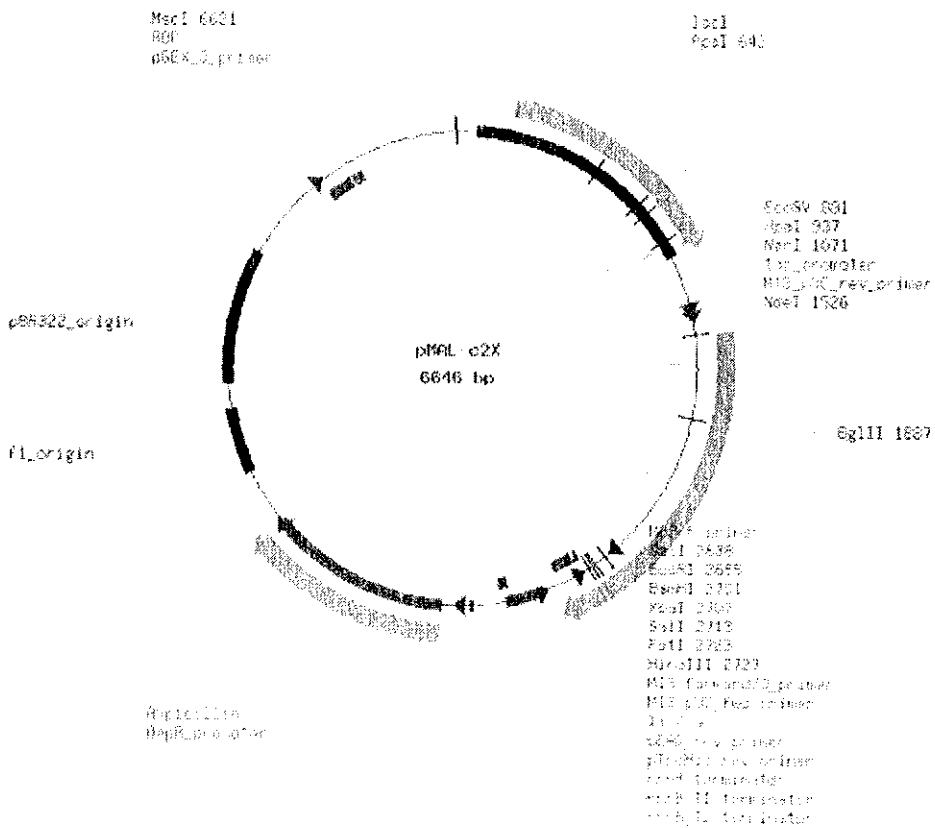
1. Kaelin, W.G. *et al. Cell* **70**, 351 (1992).

Vector Backbone: pMAL-c2X

Vendor:	New England Biolabs
Vector Type:	Bacterial
Promoter:	P-lac
Backbone Size (bp):	6700
Tag:	Maltose-binding protein, MBP (Nterm)
Bacteria Resistance:	Ampicillin
Catalog Number:	N8077S
Sequence and Map:	Sequence (Click to see features and cutters)

Comments: Maltose-binding protein fusion cleaved by Factor Xa. Goes to periplasm.

Click on map to enlarge



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MATERIAL SAFETY DATA SHEET

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Msds Revision Date: 5/08

Vector #N8076

SECTION 1 - PRODUCT

Product Name: pMAL-c2X

SECTION 2– CHEMICAL INFORMATION

1.	Tris-HCl	< 1%	Cas.	#77-86-1
2.	EDTA	< 1%	Cas.	#60-00-4

SECTION 3–HAZARDOUS IDENTIFICATION

NAME OF CHEMICAL: Factor X from Bovine Plasma Cas No. #9001-29-0 SARA 313: NO

HMIS Rating
Health: 0
Flammability: 0
Reactivity: 0

NFPA Rating
Health: 0
Flammability: 0
Reactivity: 0

SECTION 4 –FIRST AID MEASURES

ORAL EXPOSURE: If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE: If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE: In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE: In case of contact, immediately flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. 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 Hazard (s):
Emits toxic fumes under fire conditions.

Flash point: N/A

Flammability: N/A

Autoignition Temp: N/A

SECTION 6 –ACCIDENTAL RELEASE MEASURES

PROCEDURE(S) OF PERSONAL PRECAUTION(S):

Exercise appropriate precautions to minimize direct contact with skin or eyes and prevent inhalation.

METHODS FOR CLEANING UP:

Absorb on sand or vermiculite and place in closed containers for disposal.
Ventilate area and wash spill site after material pickup is complete.

Flush spill area with copious amounts of water.

SECTION 7– HANDLING AND STORAGE

Handling:

User Exposure: Avoid Inhalation.

Avoid contact with eyes, skin and clothing.

Avoid prolonged or repeated exposure.

Storage:

Keep tightly closed.

SECTION 8 – EXPOSURE CONTROLS/PPE

Engineering Controls: Safety shower and eye bath. Mechanical exhaust required.

Personal Protective Equipment:

Respiratory

Hand:
Compatible chemical-resistant gloves.

Eye:
Chemical safety goggles.

General Hygiene Measures:

Wash hands thoroughly after handling.

Wash contaminated clothing before use.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Appearance: **Physical State: Liquid**

Property	Value
Molecular Weight:	NA
pH:	NA
BP/BP Range:	NA
MP/MP Range:	NA
Freezing Point:	NA
Vapor Pressure:	NA
Vapor Density:	NA
Saturated Vapor:	NA
SG/Density:	NA
Bulk Density:	NA
Odor Threshold:	NA
Volatile %:	NA
Voc Content:	NA
Water Content:	NA
Solvent Content:	NA
Evaporation Rate:	NA
Viscosity:	NA
Surface Tension:	NA
Partition Coefficient:	NA
Decomposition Temp:	NA
Flash Point:	NA
Explosion Limits:	NA
Flammability:	NA
Autoignition Temp:	NA
Refraction Index:	NA
Optical Rotation:	NA
Miscellaneous Data:	NA
Solubility in Water:	NA

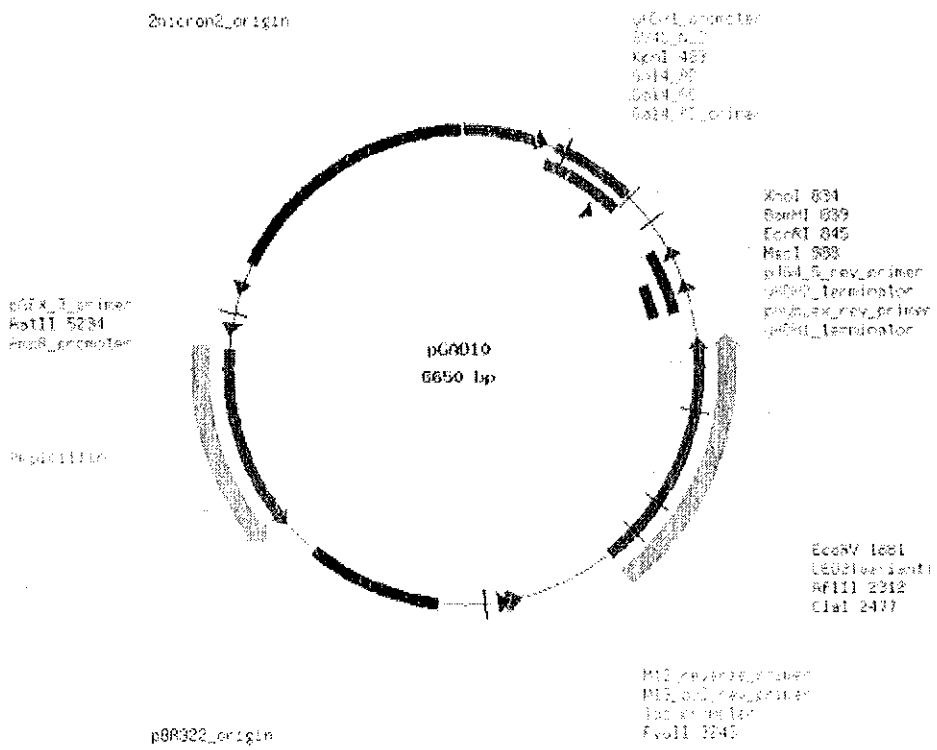
N/A = not available

Vector Backbone: pGAD10

Backbone Size (bp):	6650
GenBank Accession Number:	U13188
Sequence and Map:	Sequence (Click to see features and cutters)

Comments: NCBI gi: 532698 Hosts: E.coli. (Information source: VectorDB.)

Click on map to enlarge



Vector Backbone: pAS2

Vendor:	ATCC
Backbone Size (bp):	8500
Catalog Number:	87008

Comments: Restriction digests of the clone give the following sizes (kb): BamHI--8.6; SalI--8.6; SmaI--8.6. (ATCC staff) Shuttle expression vector used to create fusion proteins consisting of the nuclear localization sequence from SV40 T antigen, the GAL4 DNA-binding domain (aa 1-147), and a HA (hemagglutinin) epitope tag in frame with the activation domain. [1] The order of the major features in this plasmid is: ADC1 (ADH) promoter -> - GAL4 DNA binding domain - HA - NdeI/MCS/SalI - ADC1 terminator - pMB1 ori - ampR - 2 micron ori - TRP1 -> - f1 ori - <- CYH2. [2] Growth: LB plus ampicillin (ATCC medium number 1227) 37C Deposited by: Elledge S.J. Hosts: E.coli, yeast, Saccharomyces cerevisiae. (Information source: VectorDB.)