The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: August 12, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Nathalie Berube**
DEPARTMENT:	**Paediatrics and Biochemistry**
ADDRESS:	**800 Commissioners Road East**
PHONE NUMBER:	**519-685-8500x55066**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**nberube@uwo.ca**

Location of experimental work to be carried out :

Building : **LRCP/VRL** Room(s): **A4-116(lab)**

Building : Room(s):

Building : Room(s):

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR, International Rett Syndrome Foundation (IRSF)**

GRANT TITLE(S): **CIHR : Neuronal functions of the ATRX mental retardation gene**
CIHR: Control of skeletal development by the chromatin remodeling protein ATRX
IRSF: Epigenetic regulation of gene expression by MeCP2 in the mouse brain

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

Name	UWO E-mail Address	Date of Biosafety Training
Ashley Watson	**Lwatso6@uwo.ca**	**04/2009**
Kristin Kernoham	**kkernoha@uwo.ca**	**04/2011**

Mike Levey	Mlevy2@uwo.ca	04/2011
Yan Jiang	Jiangy02@yahoo.com (not UWO employ	04/2011
Lauren Solomon	Lsolomo2@uwo.ca	10/2010
Jason Bush	bush@ualberta.ca (no UWO address)	04/2011
Adrienne Elbert	aelbert@uwo.ca	07/2011
Matthew Edwards	Medwar27@uwo.ca	06/2011

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Use of Biological Agents:
Adenovirus containing cre recombinase fused to GFP will be used to delete gene sequences containing LoxP sites in mouse embronic fibroblasts. Adenovirus-GFP is used as a control.

shRNAs or siRNAs are used to deplete ATRX,CTCF and Mecp2 or in primary cells or cell lines. We have not yet deleted MeCP2 and CTCF.

Plasmids expressing various genes are amplified in bacteria. The plasmid DNA is isolated and purified and transfected in cultured cell lines.

Retroviral constructs expressing specific tagged constructs are used produce virus to deliver exogenous genes or shRNAs (to decrease expression of target genes) in mouse embryonic fibroblasts . Transfection into a package cell line produces hightiter, replication-incompetent viruses. Protein extracts obtained from the transduced cells are used for Western blot analyses, RNA is used for real-time PCR, and chromatin extracts are used for chromatin immunoprecipitation assays.

Precautions taken:

When making or using biological agents, lab coats and gloves are worn but removed before leaving the laboratory area, work surfaces are decontaminated before and after each procedure. Mechanical pipetting devices are used for all procedures. Filter tips are used when handling viruses. Hands are washed before and after handling biological agents. In general, avoid inhalation and contact with eyes, skin, and clothing.

Storage of Biological Agents: In sealed containers that are appropriately labeled

All E. coli cells with glycerol are stored at -80c.
Virus with buffer are stored at -80c.
Cell lines and MEFs are stored in 10% DMSO at -160c

Disposal of Biological Agents:

Bacterial cell cultures that harbor foreign DNA:
All solid phase media and plastics pipettes and dishes that have come in contact with bacterial cultures are disposed of in biohazardous waste containers and autoclaved. All liquid cultures are bleached and disposed of down the drain.

Mammalian cell culture (primary and immortal cell isolates):
All tissue culture plastics are disposed of in biohazardous waste containers for autoclaving. All liquid waste is bleached and disposed of down the drain.

Viral production:
All tissue culture plastics are collected in biohazardous waste bags inside the biological safety cabinet and autoclaved. All glassware is disinfected with bleach inside the biological safety cabinet. All liquid waste is bleached inside the biological safety cabinet before removal.

Extraction of DNA from mammalian tissues:
All extractions are carried out in a laminar flow hood. We use an agent from Qiagen Extraction Kit that dissolves the tissue and allows the DNA to be isolated. Its harsh chemical make up destroys any associated pathogens that may be present. Dissolved tissue is disposed of in biohazardous waste containers for autoclaving. The DNA is further purified and sterilized in alcohol and stored.

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

1. The roles of the ATRX chromatin remodeling protein in mouse brain development. We are investigating the outcome of ATRX loss of function using the Cre/loxP system in the mouse. Using various mouse Cre driver lines, we have inactivated ATRX in early neurogenesis and are determining the effects on cell division and differentiation, gene expression profiles, higher order chromatin looping, and the epigenetic state of chromatin in the developing brain. In some experiments, we will use adenovirus-cre recombinase (Ad-Cre) to inactivate ATRX in cultured mouse embryonic fibroblasts obtained from ATRX floxed mice. Cells will also be infected with a control adenovirus-Green Fluorescent Protein (Ad-GFP). Both of these adenoviral vectors are non-oncogenic and non-growth promoting. The infected cells will not be injected in animals. (Funded by CIHR)

2. The roles of the ATRX chromatin remodeling protein in mouse skeletal development. In collaboration with Dr. Frank Beier at UWO, we are using various Cre driver lines to inactivate ATRX in early limb bud mesenchyme, cartilage or osteoblasts. We are investigating the outcome of ATRX loss of function using histological, cellular and molecular techniques. (Funded by CIHR)

3. Epigenetic regulation of neuronal genes by the chromatin proteins ATRX and MeCP2. Using mouse models that lack either ATRX or MeCP2 in the brain, we are identifying genes that are bound and co-regulated by both these proteins and that may contribute to the related human syndromes, ATR-X and Rett syndrome. (Funded by IRSF)

4. Higher order chromatin looping and insulator functions during brain development. The CTCF insulator protein is a crucial factor in maintaining higher order structure of chromatin. We aim to identify CTCF-binding sites that are specific to brain tissue and to determine the importance of CTCF through conditional inactivation of the gene in the mouse brain.

Acronyms used:
ATRX: alpha-thalassemia mental retardation, X-linked
Cre: Cre recombinase
Floxed: indicates that loxP sites have been introduced in the genome
MeCP2: Methyl-CpG- binding protein 2
CTCF: CCCTC binding factor
CIHR: Canadian Institutes for Health Research
IRSF: International Rett Syndrome foundation.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species _____
What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.coli DH5a One shot Top10 completant cells	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.15 L 0.1L**	**invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
E.coli DH10B	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1L**	**TCAG Genome Resource Facility**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
adenovirus	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**0.1L**	**Other investigators**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
Retroviruses	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**0.1L**	**Other investigators**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

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	**Embryonic telencephalon tissue & MEF**	**2008-041-02**
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**Hela,MCF-7,C33A,U2OS,SH-sy5y**	**Level (2)**	**ATCC, other investigators**
Rodent	☒ Yes ☐ No	**Neuro2A**	**Level (2)**	**ATCC, other investigators**
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

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

Tissues (preserved)				

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
E.Coli DH5a, DH10b	**See accompanying table of plasmids**	**See accompanying table of plasmids**	**See table of plasmids**	**No.**	**No.**	**None**

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☒ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Adenvirus type 5 (dE1/E3)	**Ad-CMV-GFP (Cat#1060)** **Ad-CMV-CreGFP (Cat#1700)**	**Vector Biolabs**	**GFP** **CreGFP**	**None** **Deletion of ATRX. Decreased growth, DNA damage**
MMLV	**pBABE-Puro (RTV-001)**	**Cell Biolabs Inc**	**Histone H3.3-FLAG**	**Express tagged H3.3. no detectableffect on cells**
homologous to MSCV LTR	**pSUPER.retro.neo+GFP (VEC-PRT-0005/0006)**	**Oligoengine**	**Short hairpin RNAs(shRNA)**	**Depletion of ATRX. Mitotic defects**

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☒ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 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

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☐ YES ☒ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

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

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

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

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

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

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

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

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

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

11.3 What is the origin of the plant?

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

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

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

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

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

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

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Nathalie Berube ***Date:*** Oct.20,2011

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **Dec.10,2010**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:

Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician. This scenario is unlikely since we don't inject virus.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/newposition.htm

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Nathalie Berube** ***Date:*** **January 12, 2012**

15.4 Additional Comments: _____

16.0 Approvals

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

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

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

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

Special Conditions of Approval:

Dr Berube Lab

Name of Cell Line	Tumour Type	ATCC Designation	Biosafety Level	Link to MSDS
C-33A		CRM-HTB-31	1	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CRM-HTB-31&Template=cellBiology
U-2 OS		HTB-96	1	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-96&Template=cellBiology
MCF-7	Breast adenocarcinoma	HTB-22	1	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-22&Template=cellBiology
SH-SY5Y		CRL-2266	1	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CRL-2266&Template=cellBiology
Neuro-2a		CCL-131	1	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CCL-131&Template=cellBiology
Hela	cervix adenocarcinoma	CCL-2	2	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CCL-2&Template=cellBiology
HEK293		CRL-1573	2	http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CRL-1573&Template=cellBiology
Phoenix Eco Cells			2	https://www.ngvbcc.org/ReagentRepositoryDetailView.action;jsessionid=D8D9DB4D735EB24D0C777B2AC5411C3F?reagentId=68

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

M A T E R I A L S A F E T Y D A T A S H E E T

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Animal Cell Cultures at Biosafety Level 1 or 2
ATCC Catalog #: Various

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating:	Health: 0	Flammability: 0	Reactivity:0
NFPA Rating:	Health:0	Flammability:0	Reactivity:0

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

For Biosafety Level 1 Cell Cultures
Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.
This cell line is not known to cause disease in healthy adult humans. These cells have **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

See next page for Biosafety Level 2 cell cultures.

For Biosafety Level 2 Cell Cultures
Handle as a potentially biohazardous material under at least Biosafety Level 2 containment.
These cell lines are associated with human disease, hazards include: percutaneous injury, ingestion, mucous membrane exposure (U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories)**. These cells have **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

SECTION 4 - FIRST AID MEASURES

Report to your Safety Office and Seek Medical Attention as Soon as Possible

Ingestion: If person is unconscious seek emergency medical attention; never give anything by mouth to an unconscious person. If the person is conscious wash mouth out with copious amounts of water and call a physician then administer three cupfuls of water. Do not induce vomiting unless directed to do so by a physician.

Inhalation: If person is unconscious seek emergency medical attention, if person is conscious remove to fresh air and call a physician.

Dermal exposure: Immediately wash skin with copious amounts of water followed by washing with soap and copious amounts of water. Remove all contaminated clothing.

Eye exposures: Flush eyes with copious amounts of water for at least 15 minutes with eyelids separated and call a physician.

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available

Suitable Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, Halon (where regulations permit), or appropriate foam.

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent inhalation, ingestion, skin and eye contact.

Specific Hazard(s): Responders should take into consideration the biohazard risk associated with responding to a fire in the area where the material may be stored or handled.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8. Wear laboratory coat, gloves and eye protection. Avoid all contact.

Methods for Cleaning Up

Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.

Equipment/Environment: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time before clean up (30 min).

Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:
Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use fume hood or other appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

Eye Contact: Data not available. Avoid eye contact.
Skin Contact: Data not available. Avoid skin contact.
Skin Absorption: Data not available. Avoid skin absorption.
Inhalation: Data not available. Avoid inhalation.
Ingestion: Data not available. Avoid ingestion.
Parenteral Exposure: Data not available. Avoid parenteral exposure.

Sensitization
Skin: Data not available
Respiratory: Data not available

Target Organ(s) or System(s): Data not available

Signs and Symptoms of Exposure
Skin and Mucous Membranes: Data not available
Respiratory: Data not available
Gastrointestinal: Data not available

Toxicity Data: Data not available
Effects of Long Term or Repeated Exposure: Data not available
Chronic Exposure–Teratogen: Data not available
Chronic Exposure–Mutagen: Data not available
Chronic Exposure–Reproductive Hazard: Data not available

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Decontaminate all wastes before disposal (steam sterilization, chemical disinfection, and/or incineration).

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

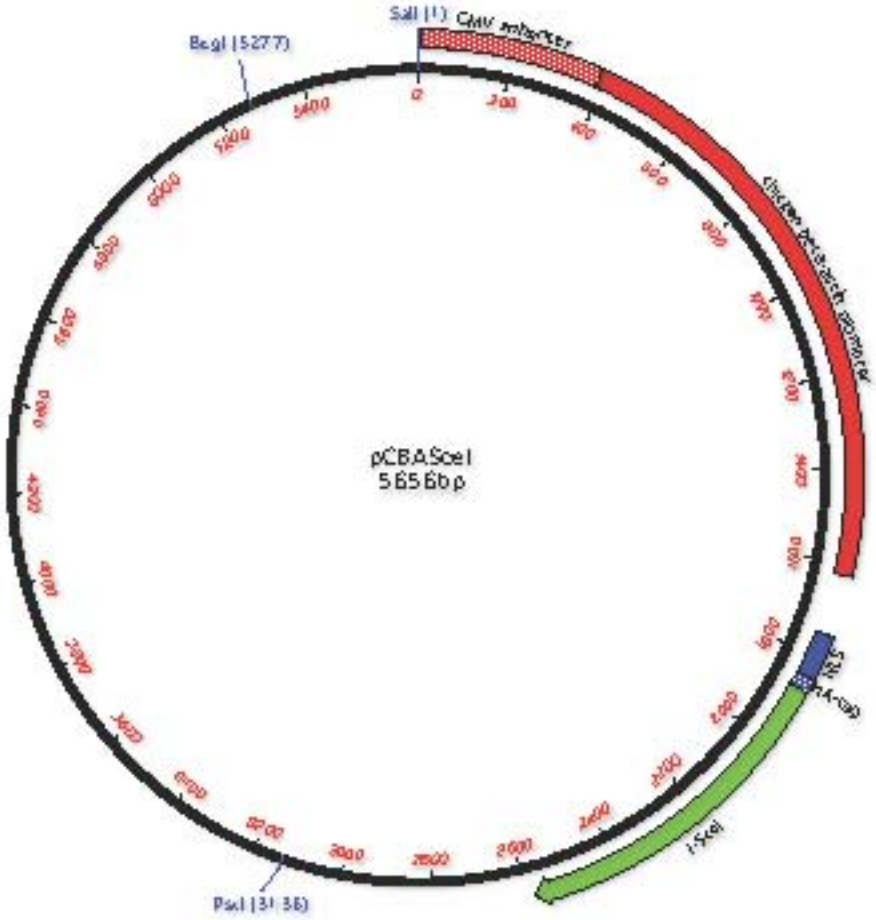
Contact ATCC for regulatory information.

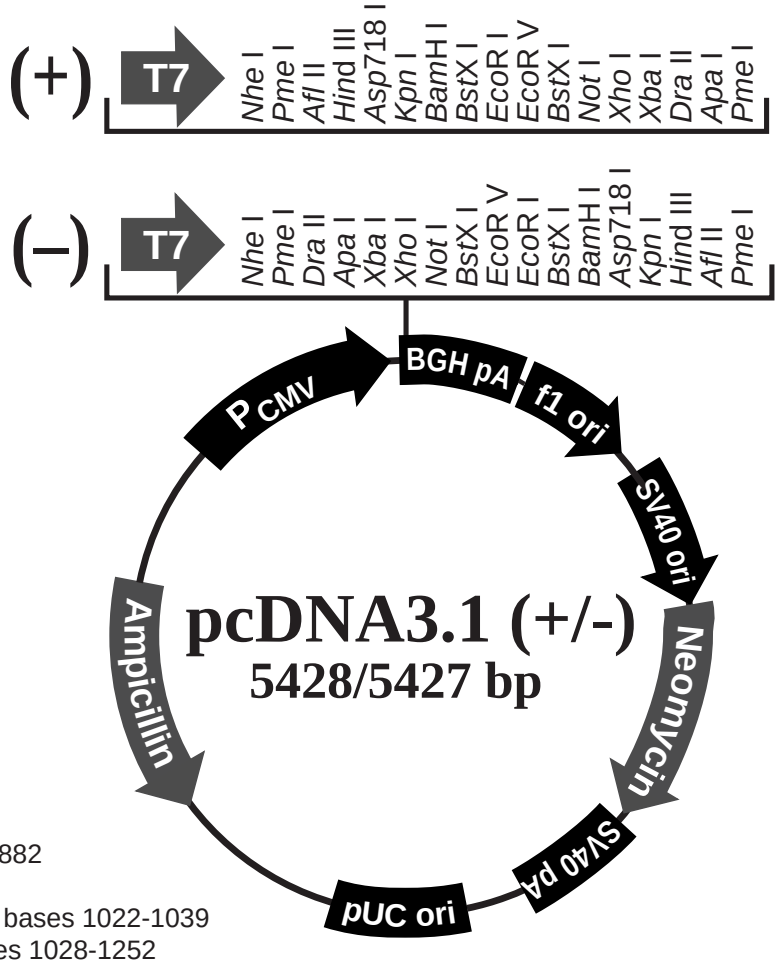
SECTION 16 - OTHER INFORMATION

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

Plasmid List – Berube Lab

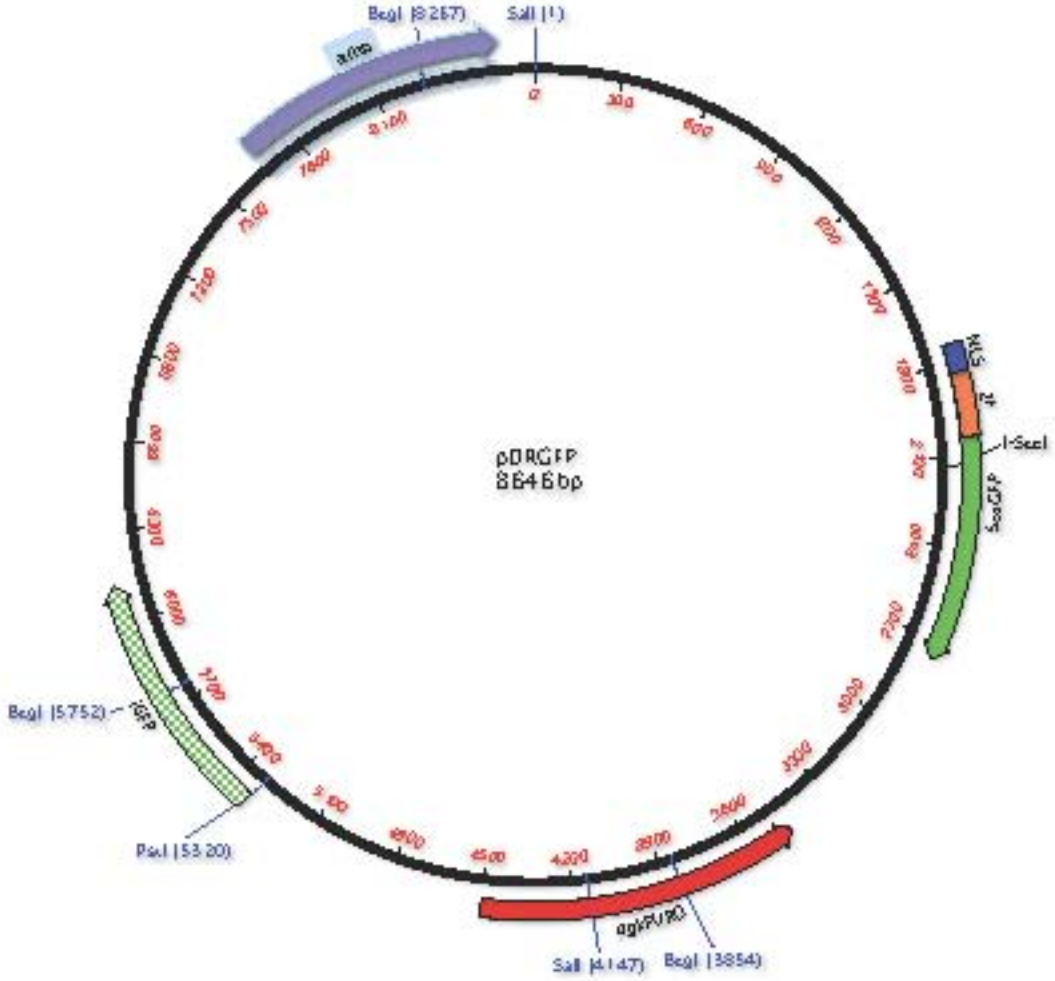
Plasmid	Source	Gene Transfected	Describe Change that Resulted
pMAL-C2	NEB	hATRX	Protein Expression Vector
psCODON	Delphi Genetics	mSMC3	Protein Expression Vector
psCODON	Delphi Genetics	mSMC3	Protein Expression Vector
psCODON	Delphi Genetics	mSMC3	Protein Expression Vector
pET30a	Novagen	mSMC3	Protein Expression Vector
pET30a	Novagen	mSMC1A	Protein Expression Vector
psCODON	Delphi Genetics	mSMC1A	Protein Expression Vector
psCODON	Delphi Genetics	mSMC1A	Protein Expression Vector
pET30a	Novagen	mSMC1A	Protein Expression Vector
pET30a	Novagen	mSTAG2	Protein Expression Vector
pET30a	Novagen	mSTAG2	Protein Expression Vector
pMAL-C2	NEB	mMecP2	Protein Expression Vector
pET30a	Novagen	mRad21	Protein Expression Vector
pMAL-C2	NEB	mRad21	Protein Expression Vector
pMAL-C2	NEB	mSororin	Protein Expression Vector
pMAL-C2	NEB	mWAPL	Protein Expression Vector
pMAL-C2	NEB	mWAPL	Protein Expression Vector
pET30a	Novagen	mWAPL	Protein Expression Vector
psCODON	Delphi Genetics	mCTCF	Protein Expression Vector
pcDNA3.1	Invitrogen	Histon3.3, GFP	Protein Expression Vector
pGEM-T	Promega		Amplify methylation DNA
pBABE-puro	other investigator		Recombination assay
pDRGFP	Addgene		Recombination assay
pCBAScel	Addgene		Check recombination
pSUPER RNAi System	Oligoengine	ATRX Si3	Amplify SiRNA
pSUPER RNAi System	Oligoengine	ATRX Si4	Amplify SiRNA
pGK-EF1aGFP	other investigator	shRNA ATRX	Amplify SiRNA
pGK-EF1aGFP	other investigator	shRNA CTCF	Amplify SiRNA
pGK-EF1aGFP	other investigator	shRNA Mecp2	Amplify SiRNA


SalI (1)
CMV enhancer
BsgI (5277)
pCBASceI
5656bp
PacI (3138)
I-SceI



Comments for pcDNA3.1 (+)
5428 nucleotides

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


BsgI (8267)
SalI (1)
amp
pDRGFP
8646bp
MCS
I-SceI
SceGFP
BsgI (5752)
IGFP
PacI (5320)
SalI (4147)
BsgI (3854)

pET-30a-c(+) Vectors

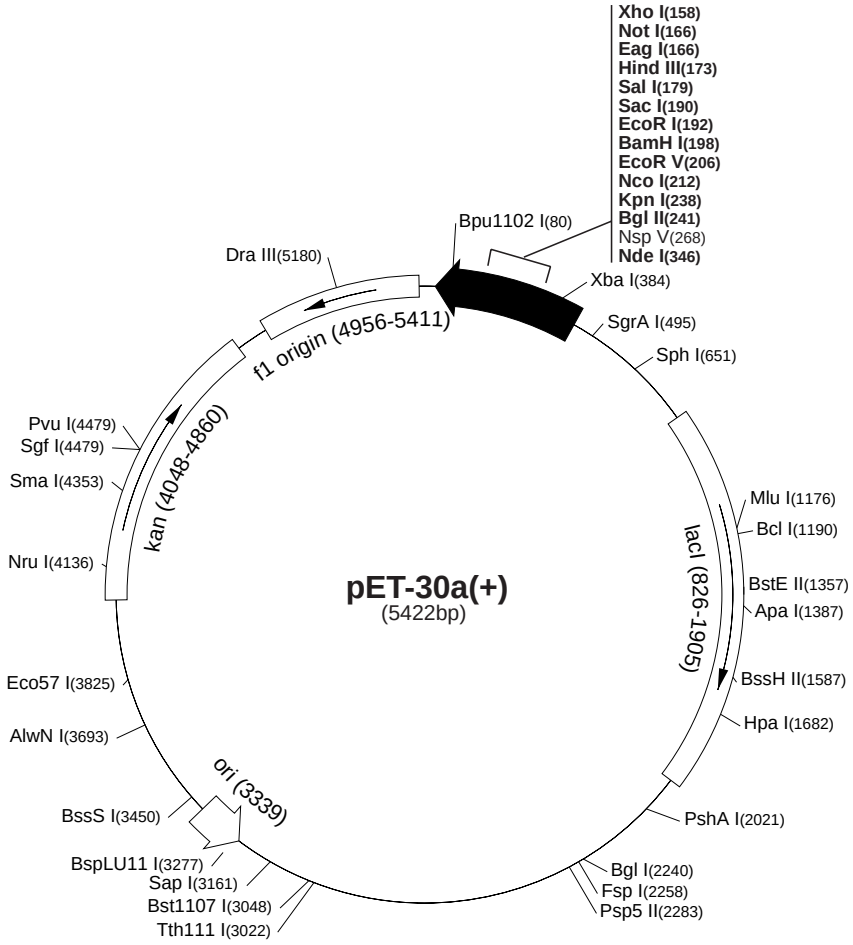
	Cat. No.
pET-30a DNA	69909-3
pET-30b DNA	69910-3
pET-30c DNA	69911-3

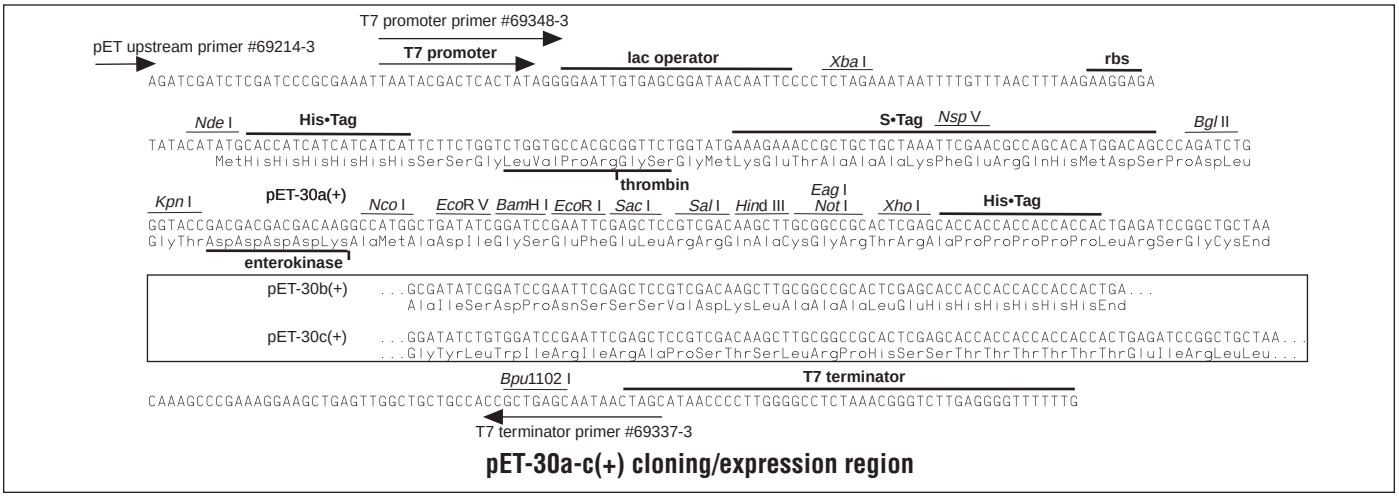
The pET-30a-c(+) vectors carry an N-terminal His•Tag®/thrombin/S•Tag™/enterokinase configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-30a(+) sequence landmarks

T7 promoter	419-435
T7 transcription start	418
His•Tag coding sequence	327-344
S•Tag coding sequence	249-293
Multiple cloning sites	
(*Nco* I - *Xho* I)	158-217
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	826-1905
pBR322 origin	3339
Kan coding sequence	4048-4860
f1 origin	4956-5411

The maps for pET-30b(+) and pET-30c(+) are the same as pET-30a(+) (shown) with the following exceptions: pET-30b(+) is a 5421bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-30c(+) is a 5423bp plasmid; add 1bp to each site beyond *Bam*H I at 198.





pET-30a-c(+) cloning/expression region

pET-30a(+) Restriction Sites

Enzyme	# Sites	Locations				
AccI	2	180	3047			
AceIII	7	943	1671	2002	2786	2927
		3229	5020			
AciI	75					
AflIII	2	1176	3277			
AluI	22					
AlwI	13					
Alw21I	7	159	190	676	1160	2271
		3095	3595			
Alw44I	3	1156	3091	3591		
AlwNI	1	3693				
ApaI	1	1387				
ApaBI	1	860				
ApoI	7	192	270	1451	4092	4276
		4982	4993			
AvaI	2	158	4351			
AvaII	5	1728	2104	2192	2283	2562
BamHI	1	198				
BanI	10	234	310	498	519	633
		1096	1815	1945	2071	5217
BanII	6	190	560	574	1387	4134
		5255				
BbsI	4	1322	1661	2035	2395	
BbvI	25					
BccI	14					
Bce83I	6	21	1990	2160	3368	3666
		3907				
BcefI	6	695	1036	1663	3779	4798
		5206				
BcgI	8	160	194	1468	1502	2002
		2036	2854	2888		
BclI	1	1190				
BfaI	6	70	385	2291	3772	4079
		5331				
BglI	1	2240				
BglII	1	241				
BmgI	1	1385				
BpmI	4	1014	1503	2137	2804	
Bpu10I	2	2383	4496			
Bpu1102I	1	80				
BsaAI	2	3029	5180			
BsaBI	3	449	459	2474		
BsaHI	5	499	520	634	1133	1816
BsaJI	10	57	212	613	619	1811
		2249	3437	4350	4351	4752
BsaWI	7	2	1495	1998	2466	3483
		3630	4614			
BsaXI	2	1835	5128			
BsbI	2	2993	5087			
BscGI	11					
BsgI	3	1027	1227	2437		
BsiI	1	3450				
BsiEI	5	169	1961	3193	3617	4479
BslI	26					
BsmI	2	4363	4440			
BsmAI	6	873	1278	1404	1791	2918
		4495				
BsmBI	3	1791	2918	4495		
BsmFI	4	637	2178	2548	5395	
BsoFI	43					
Bsp24I	10	466	498	1017	1049	1319
		1351	3770	3802	3948	3980
Bsp1286I	12					
BspEI	2	2	2466			
BspGI	1	2803				
BspLU11I	1	3277				
BsrI	21					
BsrBI	4	405	3210	4878	5324	
BsrDI	2	1223	1589			
BsrFI	7	486	495	862	2074	2234
		4433	5281			
BssHII	1	1587				

Enzyme	# Sites	Locations				
Bst1107I	1	3048				
BstEII	1	1357				
BstXI	3	978	1107	1230		
BstYI	9	132	198	241	740	1952
		2469	3918	3929	4728	
Cac8I	40					
CjeI	24					
CjePI	18					
ClaI	2	453	4170			
CviJI	85					
CviRI	31					
DdeI	11					
DpnI	23					
DraIII	1	5180				
DrdI	3	2970	3385	5135		
DrdII	2	899	5185			
DsaI	3	212	613	2249		
EaeI	4	166	484	616	1850	
EagI	1	166				
EarI	3	794	3161	4292		
EciI	3	953	3351	3497		
Eco47III	3	581	2082	2531		
Eco57I	1	3825				
EcoNI	2	711	4391			
EcoO109I	3	53	609	2283		
EcoRI	1	192				
EcoRII	9	899	1214	1754	1811	3303
		3424	3437	4367	4724	
EcoRV	1	206				
FauI	17					
FokI	9	1222	1231	2496	2558	2636
		2822	2963	4117	4723	
FspI	1	2258				
GdiII	4	166	484	616	1850	
HaeI	7	217	904	2225	3292	3303
		3755	4566			
HaeII	14					
HaeIII	24					
HgaI	11					
HgiEII	2	774	3863			
HhaI	46					
Hin4I	4	203	1075	4165	4707	
HincII	2	181	1682			
HindIII	1	173				
HinfI	18					
HpaI	1	1682				
HphI	16					
KpnI	1	238				
MaeII	14					
MaeIII	16					
MboII	13					
MluI	1	1176				
MmeI	7	3492	3676	4121	4315	4677
		4686	5157			
MnlI	25					
MseI	25					
MslI	6	1228	1516	1546	2264	2459
		2850				
MspI	29					
MspA1I	9	84	283	1206	1776	1869
		2868	2987	3619	3864	
MwoI	39					
NarI	4	499	520	634	1816	
NciI	12					
NcoI	1	212				
NdeI	1	346				
NgoAIV	4	486	2074	2234	5281	
NlaIII	26					
NlaIV	23					
NotI	1	166				
NruI	1	4136				
NsiI	2	4329	4595			

Enzyme	# Sites	Locations				
NspI	4	651	2622	2914	3281	
NspV	1	268				
Pfl1108I	1	2063				
PflMI	3	260	758	4742		
PleI	9	433	725	812	1608	3171
		3656	4711	5115	5123	
PshAI	1	2021				
Psp5II	1	2283				
Psp1406I	4	838	2206	2602	4965	
PvuI	1	4479				
PvuII	3	1776	1869	2868		
RcaI	3	574	3997	4872		
RsaI	4	236	1323	3083	4314	
SacI	1	190				
SalI	1	179				
SapI	1	3161				
Sau96I	14					
Sau3AI	23					
ScrFI	21					
SfaNI	23					
SfcI	4	418	3542	3733	5399	
SgfI	1	4479				
SgrAI	1	495				
SmaI	1	4353				
SphI	1	651				
SspI	2	4404	4972			
StyI	2	57	212			
TaqI	17					
TaqII	6	1084	1302	1975	3179	4733
		5084				
TfiI	9	1855	2157	2327	2831	3252
		4390	4446	4618	4709	
ThaI	36					
TseI	25					
Tsp45I	7	1357	2185	2716	2929	3024
		4626	5353			
Tsp509I	21					
Tth111I	1	3022				
Tth111II	8	1015	1708	2738	3867	3874
		3906	4315	4442		
UbaJI	18					
VspI	5	433	1861	1920	4678	4867
XbaI	1	384				
XcmI	3	1032	1548	1566		
XhoI	1	158				
XmnI	2	2835	4868			

Enzymes that do not cut pET-30a(+):

AatII	AflII	AgeI	AscI	AvrII
BaeI	BsaI	BseRI	BspMI	BsrGI
Bsu36I	DraI	Eam1105I	FseI	MscI
MunI	NheI	PacI	PmeI	PmlI
PstI	RleAI	RsrII	SacII	ScaI
SexAI	SfiI	SnaBI	SpeI	SrfI
Sse8387I	StuI	SunI	SwaI	

pGEM®-T and pGEM®-T Easy Vector Systems

INSTRUCTIONS FOR USE OF PRODUCTS A1360, A1380, A3600 AND A3610.

Quick PROTOCOL

Cloning PCR Products with pGEM®-T and pGEM®-T Easy Vectors

Ligation Using 2X Rapid Ligation Buffer

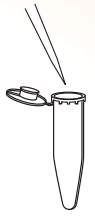
1. Briefly centrifuge the pGEM®-T or pGEM®-T Easy Vector and Control Insert DNA tubes to collect contents at the bottom of the tube.
2. Set up ligation reactions as described below. Vortex the 2X Rapid Ligation Buffer vigorously before each use. Use 0.5ml tubes known to have low DNA-binding capacity.

Reagents	Standard Reaction	Positive Control	Background Control
2X Rapid Ligation Buffer, T4 DNA Ligase	5µl	5µl	5µl
pGEM®-T or pGEM®-T Easy Vector (50ng)	1µl	1µl	1µl
PCR product	Xµl	–	–
Control Insert DNA	–	2µl	–
T4 DNA Ligase (3 Weiss units/µl)	1µl	1µl	1µl
Deionized water to a final volume of	10µl	10µl	10µl

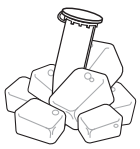
3. Mix the reactions by pipetting. Incubate the reactions 1 hour at room temperature. Alternatively, incubate the reactions overnight at 4°C for the maximum number of transformants.

Transformation of JM109 High Efficiency Competent Cells

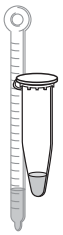
1. Prepare LB/ampicillin/IPTG/X-Gal plates.
2. Centrifuge the ligation reactions briefly. Add 2µl of each ligation reaction to a sterile 1.5ml tube on ice. Prepare a control tube with 0.1ng of uncut plasmid.
3. Place the JM109 High Efficiency Competent Cells in an ice bath until just thawed (5 minutes). Mix cells by **gently** flicking the tube.
4. Carefully transfer 50µl of cells to the ligation reaction tubes from Step 2. Use 100µl of cells for the uncut DNA control tube. **Gently** flick the tubes and incubate on ice for 20 minutes.
5. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42°C. DO NOT SHAKE. Immediately return the tubes to ice for 2 minutes.
6. Add 950µl room temperature SOC medium to the ligation reaction transformations and 900µl to the uncut DNA control tube. Incubate for 1.5 hours at 37°C with shaking (~150rpm).
7. Plate 100µl of each transformation culture onto duplicate LB/ampicillin/IPTG/X-Gal plates. For the uncut DNA control, a 1:10 dilution with SOC is recommended.
8. Incubate plates overnight at 37°C. Select white colonies.



Set up ligation. Incubate at room temperature for 1 hour or at 4°C overnight.

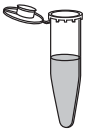


Thaw competent cells on ice. Add 50µl cells to 2µl of the ligation reaction. Incubate on ice for 20 minutes.



Heat Shock for 45–50 seconds at exactly 42°C.

Incubate on ice for 2 minutes.



Add SOC medium. Incubate for 1.5 hours at 37°C with shaking.

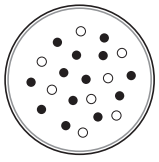


Plate. Select white colonies.

Additional protocol information in Technical Manual #TM042, available online at: **www.promega.com**

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Promega

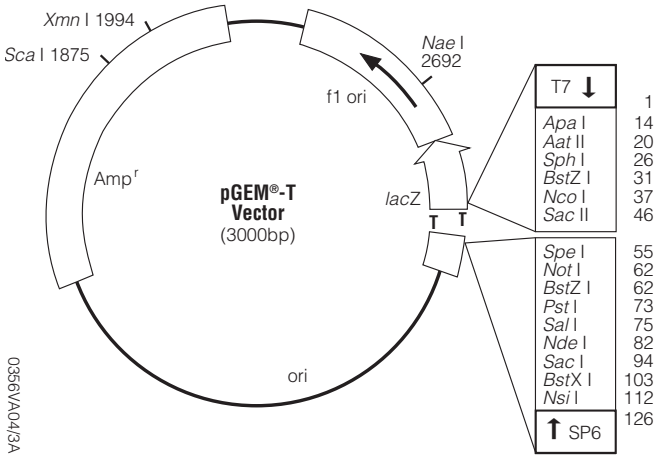
Printed in USA. Revised 3/09.
Part# 9FB033

pGEM®-T and pGEM®-T Easy Vector Systems

INSTRUCTIONS FOR USE OF PRODUCTS A1360, A1380, A3600 AND A3610.

Quick PROTOCOL

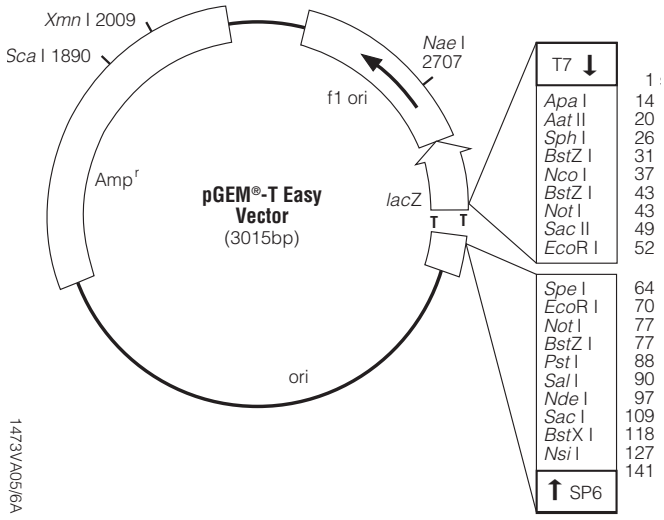
pGEM®-T Vector Circle Map and Sequence Reference Points



pGEM®-T Vector Sequence reference points:

T7 RNA Polymerase transcription initiation site	1
multiple cloning region	10–113
SP6 RNA Polymerase promoter (–17 to +3)	124–143
SP6 RNA Polymerase transcription initiation site	126
pUC/M13 Reverse Sequencing Primer binding site	161–177
lacZ start codon	165
lac operator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
lac operon sequences	2821–2981, 151–380
pUC/M13 Forward Sequencing Primer binding site	2941–2957
T7 RNA Polymerase promoter (–17 to +3)	2984–3

pGEM®-T Easy Vector Circle Map and Sequence Reference Points



pGEM®-T Easy Vector Sequence reference points:

T7 RNA Polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA Polymerase promoter (–17 to +3)	139–158
SP6 RNA Polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180
lac operator	200–216
ß-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA Polymerase promoter (–17 to +3)	2999-3

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

Printed in USA. Revised 3/09.

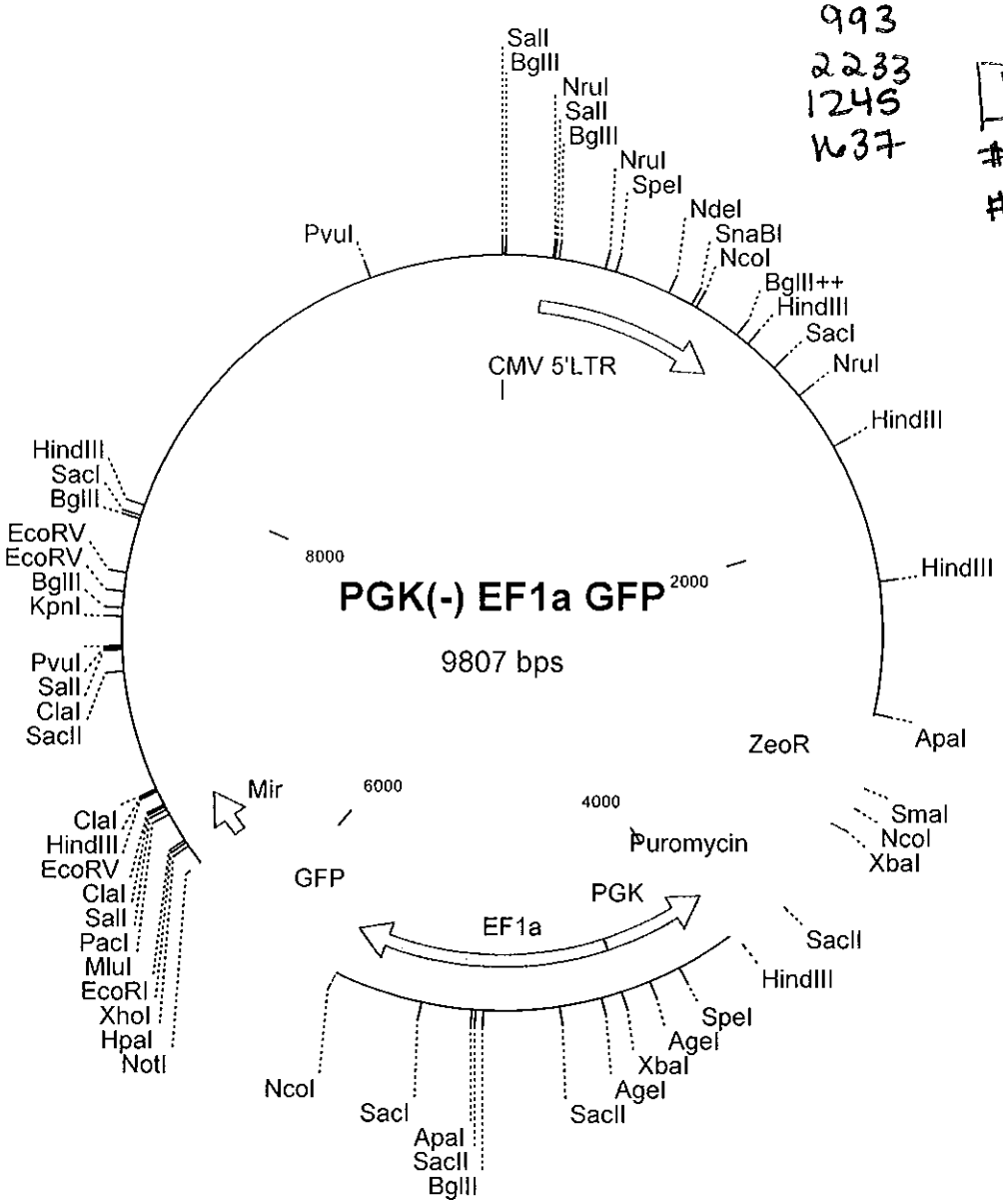
Part# 9FB033

KK shRNA → glycerol stocks

ATRX - 4 plasmids
6422 - plate 6 c 2 Mouse
5099 - mouse
3838 - mouse + human
6537 - human

CTCF - 5 plasmids
* all mouse
202
993
2233
1245
437

MeCP2
#626 Mouse
#503 rat + mouse



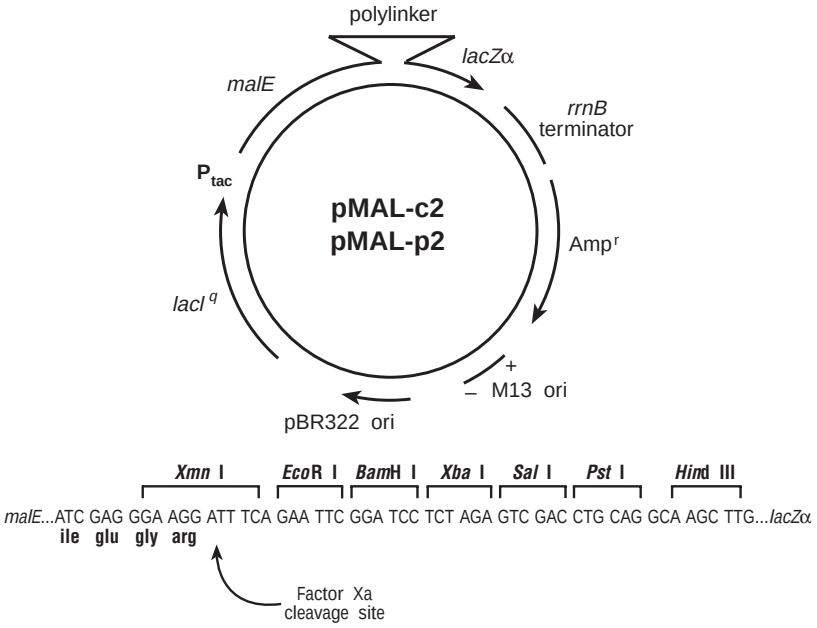


Figure 1. pMAL™-2 Vectors. pMAL™-c2 (6646 base pairs) has an exact deletion of the *malE* signal sequence. pMAL™-p2 (6721 base pairs) includes the *malE* signal sequence. Arrows indicate the direction of transcription. Unique restriction sites are indicated.

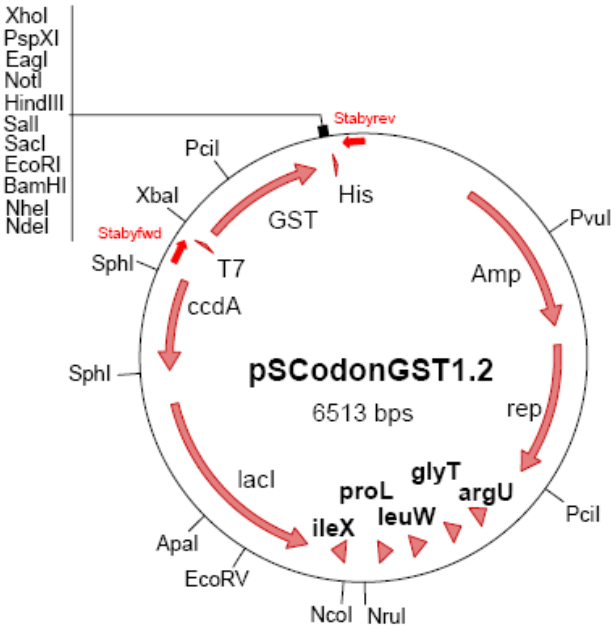


Figure 3: Restriction map of the pSCodonGST1.2 vector

Features:
-Staby forward primer: 5474-5492
-T7 promoter: 5514-5530
-GST: 5603-6256
-His: 6357-6374
-Staby reverse primer: 6417-6399(C)

The complete sequence of the vector is available on our website (www.delphigenetics.com)

MATERIAL SAFETY DATA SHEET

Section 1. Identification

Product Name: PLASMID DNA
Chemical Name: Deoxyribonucleic Acid
CAS Number:
Substance: deoxyribonucleic acid

Other Names or Code Numbers: N/A

Section 2. Composition / Information on Ingredients Section 4. First Aid Measures

Components	% Optional	OSHA PEL	ACGIH TLV	OTHER STANDARDS
PLASMID DNA	None	None	None	None

Section 3. Hazards Identification

General Statement: PLASMID is a nucleic acid prepared from normal human tissue culture cells.

Carcinogen Status: OSHA: No NTP: No IARC: No

Carcinogen Statement: No carcinogenicity data for PLASMID DNA are available in animals or humans.

Mutagenic Effects: No data available.

Teratogenic Effects: No data available.

Reproductive Effects: No data available.

Neurotoxic Effects: No data available.

Section 4. First Aid Measures

If Inhaled: Remove to fresh air. Get medical attention. If breathing has stopped, give artificial respiration. Treat symptomatically and supportively.

If Swallowed: Wash out with water.

In Case of Skin or Eye Contact: No data available. May cause irritation; flush with copious amounts of freely flowing running water. If irritation persists, seek medical care.

Skin Absorption: Limited available data indicates that Plasmid DNA is not absorbed across intact skin.

If Injected: No data available

Medical Conditions Aggravated by Exposure: No data available.

Section 5. Fire Fighting Measures

Flash Point: No data available
Flammable Limits: n/a
Extinguishing Media: Use water spray, CO2, ABC dry chemical or foam.
Special Fire Fighting Materials: No special procedures.
Unusual Fire and Explosion Hazards: No data available.

Section 6. Accidental Release Measures

Plasmid DNA is not a hazardous material as defined by the U.S. EPA. No data available. Wear gloves to clean up a spill. No other special procedures should be necessary.

Section 7. Handling and Storage

No special safety precautions are required. For product quality assurance, vials must be stored in a 2 - 8 °C (36 - 46 °F) refrigerator. Should refrigeration be unavailable, Plasmid DNA can be stored at 25 °C (77 ° F) for a period of up to 30 days. DO NOT EXPOSE TO HIGH TEMPERATURES.

Section 8. Exposure Control / Personal Protection

Wear gloves, lab coat, and safety glasses to prevent skin and eye contact.

Section 9. Physical and Chemical Properties

Molecular Formula: Deoxyribonucleic acid
Molecular Weight: Varies
Appearance/Odor: white/tan fibers
Solubilities: Soluble in water
Boiling Point: Not determined
Melting Point: Not determined
Vapor Pressure (mm HG): N/A
Vapor Density (Air = 1): N/A
Specific Gravity (H(2)0 = 1): N/A
pH: See data sheet

Section 10. Stability and Reactivity

Plasmid DNA is Stable
Hazardous Polymerization: Will Not Occur
Incompatible Materials: No data available
Conditions to Avoid: No special safety precautions required.
Hazard Decomposition Products: No data available.

MATERIAL SAFETY DATA SHEET

Section 11. Toxicology Information

THE CHEMICAL, PHYSICAL AND TOXICOLOGICAL PROPERTIES OF PLASMID DNA HAVE NOT BEEN THOROUGHLY INVESTIGATED.

Refer to Section 3.

Section 12. Ecological Information

No data available. Plasmid DNA is not a regulated hazardous material.

Section 13. Disposal Considerations

Plasmid DNA is not a regulated hazardous material. Follow federal, state and local environmental regulations for disposal of prescription drugs.

Section 14. Transport Information

DOT Proper Shipping Name: n/a
Hazard Class: n/a
ID #: n/a

Section 15. Regulatory Information

MSDS Created: November 22, 2006
Revised: July 16, 2008
Prepared By: Richard G. Smith
Quality Assurance

DISCLAIMER: The above mentioned data are based on Lofstrand's best present knowledge of this product. Lofstrand cannot guarantee completeness or accuracy of the information contained herein, and disclaims all liability for incompleteness or inaccuracy of the information and for any claims of damages arising from handling or use of this product.

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

Product code 350492
Product name pcDNA3.1/CAT

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

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

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

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. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

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

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

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. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
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 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

Principle Routes of Exposure/ Potential Health effects

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

Ingestion May be harmful if swallowed.

Specific effects **(Long Term 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
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

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

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company 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, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

addgene

One Kendall Square, Building 600 3rd Flr, Cambridge, MA 02139
info@addgene.org • Tel: 617-225-9000 • Fax: 888-734-0533

MATERIAL SAFETY DATA SHEET
E. coli bacteria with plasmids

Last Updated: 03/10/2006
Version 1.02

SECTION 1: PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME:	**E. coli bacteria with plasmids**
COMPANY:	**Addgene**
STREET ADDRESS:	**One Kendall Square, Building 600, 3rd Floor**
CITY, STATE, ZIP, COUNTRY:	**Cambridge, MA 02139 US**
TELEPHONE:	**(617) 225-9000**
FAX:	**(888) 734-0533**
PRODUCT USE:	**For laboratory research use only.**

SECTION 2: COMPOSITION/INFORMATION ON INGREDIENTS

SUBSTANCE:	**E. coli bacteria**
CAS NO.	**None**
SARA 313 REPORTABLE	**No**

SECTION 3: HAZARDS IDENTIFICATION

ROUTES OF ENTRY:	**Skin or eye contact**
POTENTIAL HEALTH EFFECTS	
EYES:	**May cause irritation of the eye**
SKIN:	**May cause irritation of the skin.**
INGESTION:	**May cause nausea or vomiting.**
INHALATION:	**No toxicity expected from inhalation.**
CARCINOGENICITY	**Not listed by OSHA, IARC, or NTP**

SECTION 4: FIRST AID MEASURES

EYES:	**Rinse opened eyes for at least 15 minutes. If eyes become irritated, get medical attention.**
SKIN:	**Wash skin with soap and water.**
INGESTION:	**Rinse mouth. If irritation or symptoms occur, get medical attention.**
INHALATION:	**Find fresh air. If symptoms occur, get medical attention.**

SECTION 5: FIRE-FIGHTING MEASURES

EXTINGUISHING MEDIA:	**Water, carbon dioxide, dry chemical powder, or foam**
SPECIAL PROCEDURES:	**Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.**

SECTION 6: ACCIDENTAL RELEASE MEASURES

ENVIRONMENTAL PRECAUTIONS: Keep out of sewers and waterways.

CLEAN-UP METHODS: Clean spills with chlorine bleach or 70% ethanol. Autoclave materials and dispose of waste in accordance with federal, state, and local safety regulations.

SECTION 7: HANDLING AND STORAGE

HANDLING: Avoid inhalation and contact with eyes, skin, and clothing.

STORAGE: Store at 4°C. Create frozen stocks for long-term storage.

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS: Safety shower and eye bath

VENTILATION : General ventilation

RESPIRATORY PROTECTION: Not required

EYE PROTECTION: Safety goggles

SKIN PROTECTION: Protective gloves

WORK HYGIENIC PRACTICES: Use safe laboratory practices. Handle as biohazard material.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

FORM: Bacteria supplied in glass vial with LB agar.

ODOR: Odorless

COLOR: Colorless

SECTION 10: STABILITY AND REACTIVITY

STABILITY: Stable

CONDITIONS TO AVOID: None known

MATERIALS TO AVOID: None known

HAZARDOUS DECOMPOSITION OR BY-PRODUCTS: None known

HAZARDOUS POLYMERIZATION: None

SECTION 11: TOXICOLOGICAL INFORMATION

TOXICOLOGICAL INFORMATION: May be irritating to eyes and skin.

SECTION 12: ECOLOGICAL INFORMATION

ECOLOGICAL INFORMATION: No data.

SECTION 13: DISPOSAL CONSIDERATIONS

WASTE DISPOSAL METHOD: Observe all federal, state, and local laws and regulations.

RCRA HAZARD CLASS: Not applicable.

SECTION 14: TRANSPORT INFORMATION

U.S. DEPARTMENT OF TRANSPORTATION
HAZARD CLASS: Non-hazardous

WATER TRANSPORTATION
HAZARD CLASS: Non-hazardous

AIR TRANSPORTATION
HAZARD CLASS: Non-hazardous

SECTION 15: REGULATORY INFORMATION

OSHA REGULATORY STATUS (29 CFR 1910.1200): Irritant

TSCA: No specific regulations

CERCLA: None

SARA TITLE III: None

SARA 311/312 HAZARD CATEGORIES: None

SARA 313 REPORTABLE INGREDIENTS: None

STATE REGULATIONS: N/A

SECTION 16: OTHER INFORMATION

DISCLAIMER: For R&D use only. Not for drug, household or other uses.

The above information is correct to the best of our knowledge, but does not purport to be all inclusive and shall be used only as a guide. Addgene shall not be held liable for any damage resulting from handling or from contact with the above product.

Kit components

Product pGEM®-T Easy Vector System I
Product code A1360

Substance number	Description	Amount	Symbols
A137	pGEM®-T Easy	1	-
A363	Control Insert DNA	1	-
M180	T4 DNA Ligase	1	-
C671	2X Rapid Ligation Buffer, T4 DNA Ligase	1	-

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

*
1 Identification of the substance/mixture and of the company/undertaking

Product identifier

Trade name: pGEM®-T Easy

Article number: *A137*
Application of the substance / the preparation *Laboratory chemicals*

Details of the supplier of the safety data sheet
Manufacturer/Supplier:

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
U.S.A.
1-800-356-9526 or (608)-274-4330

Information department: *MSDS author: Regulatory.Affairs@promega.com*
Emergency telephone number:
For Chemical Emergency ONLY (spill, leak, fire, exposure or accident), call CHEMTREC at 1-800-424-9300
For call originating outside the United States dial 001-703-527-3887

2 Composition/information on ingredients

Chemical characterization: Mixtures
Description: *Mixture of the substances listed below with nonhazardous additions.*

Dangerous components: *Void*
Additional information: *For the wording of the listed risk phrases refer to section 15.*

*
3 Hazards identification

Classification of the substance or mixture

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Not applicable. Product has been classified as non-hazardous.
Information concerning particular hazards for human and environment:
The product does not have to be labelled due to the calculation procedure of international guidelines.
Classification system:
The classification was made according to the latest editions of international substances lists, and is expanded upon by company and technical literature data.

Label elements

Labelling according to EU guidelines:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

(Contd. on page 2)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

Trade name: pGEM®-T Easy

(Contd. of page 1)

Classification system:
NFPA ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
HMIS-ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
OSHA Hazard Overview (Criteria according to 29CFR1910.1200): *Not applicable*
Target Organ(s): *Not applicable or unknown*

*
4 First aid measures

General information: *No special measures required.*
After inhalation: *Supply fresh air; consult doctor in case of complaints.*
After skin contact: *Generally the product does not irritate the skin.*
After eye contact: *Rinse opened eye for several minutes under running water.*
After swallowing: *If symptoms persist consult doctor.*

*
5 Firefighting measures

Suitable extinguishing agents:
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Special hazards arising from the substance or mixture *None known*
Protective equipment: *No special measures required.*

*
6 Accidental release measures

Personal precautions, protective equipment and emergency procedures *Not required.*
Environmental precautions: *No special measures required.*
Methods and material for containment and cleaning up:
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Reference to other sections
No dangerous substances are released.
See Section 7 for information on safe handling.
See Section 13 for disposal information.

*
7 Handling and storage

Handling:
Precautions for safe handling *No special measures required.*
Information about protection against explosions and fires: *The product is not flammable.*
Storage:
Requirements to be met by storerooms and receptacles: *No special requirements.*
Information about storage in one common storage facility: *Not required.*
Further information about storage conditions: *None.*
Specific end use(s) *No further relevant information available.*

(Contd. on page 3)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: pGEM®-T Easy

(Contd. of page 2)

8 Exposure controls/personal protection

Components with limit values that require monitoring at the workplace:
The product does not contain any relevant quantities of materials with critical values that have to be monitored at the workplace.
Additional information: The lists that were valid during the creation were used as basis.
Personal protective equipment:
General protective and hygienic measures:
The usual precautionary measures for handling chemicals should be followed.
Breathing equipment: Not required.
Protection of hands:
Protective gloves
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
Material of gloves
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer. As the product is a preparation of several substances, the resistance of the glove material can not be calculated in advance and has therefore to be checked prior to the application.
Eye protection: Goggles recommended during refilling.

9 Physical and chemical properties

General Information	
Appearance:	
Form:	Fluid
Color:	Colorless
Odor:	Characteristic
Odour threshold:	Not determined.
pH-value at 20°C (68 °F):	7.4
Change in condition	
Melting point/Melting range:	0°C (32 °F)
Boiling point/Boiling range:	100°C (212 °F)
Flash point:	Not applicable.
Flammability (solid, gaseous):	Not applicable.
Ignition temperature:	
Decomposition temperature:	Not determined.
Auto igniting:	Product is not selfigniting.
Danger of explosion:	Product does not present an explosion hazard.
Explosion limits:	
Lower:	Not determined.
Upper:	Not determined.
Vapor pressure:	Not determined.
Density:	Not determined.
Relative density	Not determined.
Vapour density	Not determined.
Evaporation rate	Not determined.

(Contd. on page 4)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: pGEM®-T Easy

(Contd. of page 3)

Solubility in / Miscibility with	
Water:	Not miscible or difficult to mix.
Segregation coefficient (n-octonol/water):	Not determined.
Viscosity:	
Dynamic:	Not determined.
Kinematic:	Not determined.
Solvent content:	
Organic solvents:	0.0 %
Water:	99.9 %
Solids content:	0.1 %
Other information	No further relevant information available.

10 Stability and reactivity

Thermal decomposition / conditions to be avoided: No decomposition if used according to specifications.
Incompatible materials: No further relevant information available.
Hazardous decomposition products: No dangerous decomposition products known.

11 Toxicological information

Acute toxicity:
LD/LC50 values that are relevant for classification: No data available
Primary irritant effect:
on the skin: No irritant effect.
on the eye: Irritating effect.
Sensitization: No sensitizing effects known.
Additional toxicological information:
The product is not subject to classification according to internally approved calculation methods for preparations:
When used and handled according to specifications, the product does not have any harmful effects according to our experience and the information provided to us.

*
12 Ecological information

Aquatic toxicity: Not harmful to the aquatic environment
Persistence and degradability Not available
Behavior in environmental systems:
Bioaccumulative potential Not known
Ecotoxical effects:
Remark: Not available
Additional ecological information:
General notes: Generally not hazardous for water

(Contd. on page 5)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: pGEM®-T Easy

(Contd. of page 4)

*
13 Disposal considerations

Waste treatment methods
Recommendation:
Disposal should be in accordance with applicable regional, national and local laws and regulations.
Refer to Section 7: Handling and Storage and Section 8: Exposure Control/Personal Protection for additional handling information and protection of employees.

Uncleaned packagings:
Recommendation: Disposal must be made according to official regulations.

*
14 Transport information

Contact Promega Safety Department for additional transportation information

DOT regulations:	Not regulated
Hazard class:	-
Land transport ADR/RID (cross-border):	Not regulated
ADR/RID class:	-
UN-Number:	Not regulated
Maritime transport IMDG:	
IMDG Class:	-
Marine pollutant:	No
Air transport ICAO-TI and IATA-DGR:	Not regulated
ICAO/IATA Class:	-

*
15 Regulatory information

Sara

Section 355 (extremely hazardous substances):
None of the ingredients are listed.

Section 313 (Specific toxic chemical listings):
None of the ingredients are listed.

TSCA (Toxic Substances Control Act):
All ingredients are listed.

Proposition 65

Chemicals known to cause cancer:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for females:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for males:
None of the ingredients are listed.

Chemicals known to cause developmental toxicity:
None of the ingredients are listed.

(Contd. on page 6)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: pGEM®-T Easy

(Contd. of page 5)

Cancerogenity categories

EPA (Environmental Protection Agency)
None of the ingredients are listed.

IARC (International Agency for Research on Cancer)
None of the ingredients are listed.

NTP (National Toxicology Program)
None of the ingredients are listed.

TLV (Threshold Limit Value established by ACGIH)
None of the ingredients are listed.

MAK (German Maximum Workplace Concentration)
None of the ingredients are listed.

NIOSH-Ca (National Institute for Occupational Safety and Health)
None of the ingredients are listed.

OSHA-Ca (Occupational Safety & Health Administration)
None of the ingredients are listed.

Product related hazard informations:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

National regulations:

Water hazard class: Generally not hazardous for water.

*
16 Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Department issuing MSDS:
Promega Corporation
Environmental Health and Safety Department
2800 Woods Hollow Road
Madison, WI
Ph:(608)274-4330
*** Data compared to the previous version altered.**

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

*
1 Identification of the substance/mixture and of the company/undertaking

Product identifier

Trade name: Control Insert DNA

Article number: *A363*
Application of the substance / the preparation *Laboratory chemicals*

Details of the supplier of the safety data sheet
Manufacturer/Supplier:

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
U.S.A.
1-800-356-9526 or (608)-274-4330

Information department: *MSDS author: Regulatory.Affairs@promega.com*
Emergency telephone number:
For Chemical Emergency ONLY (spill, leak, fire, exposure or accident), call CHEMTREC at 1-800-424-9300
For call originating outside the United States dial 001-703-527-3887

2 Composition/information on ingredients

Chemical characterization: Mixtures
Description: *Mixture of the substances listed below with nonhazardous additions.*

Dangerous components: *Void*
Additional information: *For the wording of the listed risk phrases refer to section 15.*

*
3 Hazards identification

Classification of the substance or mixture

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Not applicable. Product has been classified as non-hazardous.
Information concerning particular hazards for human and environment:
The product does not have to be labelled due to the calculation procedure of international guidelines.
Classification system:
The classification was made according to the latest editions of international substances lists, and is expanded upon by company and technical literature data.

Label elements

Labelling according to EU guidelines:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

(Contd. on page 2)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

Trade name: Control Insert DNA

(Contd. of page 1)

Classification system:
NFPA ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
HMIS-ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
OSHA Hazard Overview (Criteria according to 29CFR1910.1200): *Not applicable*
Target Organ(s): *Not applicable or unknown*

*
4 First aid measures

General information: *No special measures required.*
After inhalation: *Supply fresh air; consult doctor in case of complaints.*
After skin contact: *Generally the product does not irritate the skin.*
After eye contact: *Rinse opened eye for several minutes under running water.*
After swallowing: *If symptoms persist consult doctor.*

*
5 Firefighting measures

Suitable extinguishing agents:
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Special hazards arising from the substance or mixture *None known*
Protective equipment: *No special measures required.*

*
6 Accidental release measures

Personal precautions, protective equipment and emergency procedures *Not required.*
Environmental precautions: *Dilute with plenty of water.*
Methods and material for containment and cleaning up:
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Reference to other sections
No dangerous substances are released.
See Section 7 for information on safe handling.
See Section 13 for disposal information.

*
7 Handling and storage

Handling:
Precautions for safe handling *No special measures required.*
Information about protection against explosions and fires: *The product is not flammable.*
Storage:
Requirements to be met by storerooms and receptacles: *No special requirements.*
Information about storage in one common storage facility: *Not required.*
Further information about storage conditions: *None.*
Specific end use(s) *No further relevant information available.*

(Contd. on page 3)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: Control Insert DNA

(Contd. of page 2)

8 Exposure controls/personal protection

Components with limit values that require monitoring at the workplace:
The product does not contain any relevant quantities of materials with critical values that have to be monitored at the workplace.
Additional information: The lists that were valid during the creation were used as basis.
Personal protective equipment:
General protective and hygienic measures:
The usual precautionary measures for handling chemicals should be followed.
Breathing equipment: Not required.
Protection of hands:
Protective gloves
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
Material of gloves
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer. As the product is a preparation of several substances, the resistance of the glove material can not be calculated in advance and has therefore to be checked prior to the application.
Eye protection: Goggles recommended during refilling.

9 Physical and chemical properties

General Information	
Appearance:	
Form:	Fluid
Color:	Colorless
Odor:	Characteristic
Odour threshold:	Not determined.
pH-value at 20°C (68 °F):	7.4
Change in condition	
Melting point/Melting range:	0°C (32 °F)
Boiling point/Boiling range:	100°C (212 °F)
Flash point:	Not applicable.
Flammability (solid, gaseous):	Not applicable.
Ignition temperature:	
Decomposition temperature:	Not determined.
Auto igniting:	Product is not selfigniting.
Danger of explosion:	Product does not present an explosion hazard.
Explosion limits:	
Lower:	Not determined.
Upper:	Not determined.
Vapor pressure:	Not determined.
Density:	Not determined.
Relative density	Not determined.
Vapour density	Not determined.
Evaporation rate	Not determined.

(Contd. on page 4)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: Control Insert DNA

(Contd. of page 3)

Solubility in / Miscibility with	
Water:	Fully miscible.
Segregation coefficient (n-octonol/water):	Not determined.
Viscosity:	
Dynamic:	Not determined.
Kinematic:	Not determined.
Solvent content:	
Organic solvents:	0.0 %
Water:	99.1 %
Solids content:	0.1 %
Other information	No further relevant information available.

10 Stability and reactivity

Thermal decomposition / conditions to be avoided: No decomposition if used according to specifications.
Incompatible materials: No further relevant information available.
Hazardous decomposition products: No dangerous decomposition products known.

11 Toxicological information

Acute toxicity:
LD/LC50 values that are relevant for classification: No data available
Primary irritant effect:
on the skin: No irritant effect.
on the eye: Irritating effect.
Sensitization: No sensitizing effects known.
Additional toxicological information:
The product is not subject to classification according to internally approved calculation methods for preparations:
When used and handled according to specifications, the product does not have any harmful effects according to our experience and the information provided to us.

*
12 Ecological information

Aquatic toxicity: Not harmful to the aquatic environment
Persistence and degradability Not available
Behavior in environmental systems:
Bioaccumulative potential Not known
Ecotoxical effects:
Remark: Not available
Additional ecological information:
General notes: Generally not hazardous for water

(Contd. on page 5)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: Control Insert DNA

(Contd. of page 4)

*
13 Disposal considerations

Waste treatment methods
Recommendation:
Disposal should be in accordance with applicable regional, national and local laws and regulations.
Refer to Section 7: Handling and Storage and Section 8: Exposure Control/Personal Protection for additional handling information and protection of employees.

Uncleaned packagings:
Recommendation: Disposal must be made according to official regulations.
Recommended cleansing agent: Water, if necessary with cleansing agents.

*
14 Transport information

Contact Promega Safety Department for additional transportation information

DOT regulations:	Not regulated
Hazard class:	-
Land transport ADR/RID (cross-border):	Not regulated
ADR/RID class:	-
UN-Number:	Not regulated
Maritime transport IMDG:	
IMDG Class:	-
Marine pollutant:	No
Air transport ICAO-TI and IATA-DGR:	Not regulated
ICAO/IATA Class:	-

*
15 Regulatory information

Sara

Section 355 (extremely hazardous substances):
None of the ingredients are listed.

Section 313 (Specific toxic chemical listings):
None of the ingredients are listed.

TSCA (Toxic Substances Control Act):
All ingredients are listed.

Proposition 65

Chemicals known to cause cancer:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for females:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for males:
None of the ingredients are listed.

Chemicals known to cause developmental toxicity:
None of the ingredients are listed.

(Contd. on page 6)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: Control Insert DNA

(Contd. of page 5)

Cancerogenity categories

EPA (Environmental Protection Agency)
None of the ingredients are listed.

IARC (International Agency for Research on Cancer)
None of the ingredients are listed.

NTP (National Toxicology Program)
None of the ingredients are listed.

TLV (Threshold Limit Value established by ACGIH)
None of the ingredients are listed.

MAK (German Maximum Workplace Concentration)
None of the ingredients are listed.

NIOSH-Ca (National Institute for Occupational Safety and Health)
None of the ingredients are listed.

OSHA-Ca (Occupational Safety & Health Administration)
None of the ingredients are listed.

Product related hazard informations:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

National regulations:

Water hazard class: Generally not hazardous for water.

*
16 Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Department issuing MSDS:
Promega Corporation
Environmental Health and Safety Department
2800 Woods Hollow Road
Madison, WI
Ph:(608)274-4330
*** Data compared to the previous version altered.**

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

*
1 Identification of the substance/mixture and of the company/undertaking

Product identifier

Trade name: T4 DNA Ligase

Article number: *M180*
Application of the substance / the preparation *Laboratory chemicals*

Details of the supplier of the safety data sheet
Manufacturer/Supplier:

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
U.S.A.
1-800-356-9526 or (608)-274-4330

Information department: *MSDS author: Regulatory.Affairs@promega.com*
Emergency telephone number:
For Chemical Emergency ONLY (spill, leak, fire, exposure or accident), call CHEMTREC at 1-800-424-9300
For call originating outside the United States dial 001-703-527-3887

2 Composition/information on ingredients

Chemical characterization: Mixtures
Description: *Mixture of the substances listed below with nonhazardous additions.*

Dangerous components:		
56-81-5	*glycerol*	*25-50%*

Additional information: *For the wording of the listed risk phrases refer to section 15.*

*
3 Hazards identification

Classification of the substance or mixture

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Not applicable. Product has been classified as non-hazardous.
Information concerning particular hazards for human and environment:
The product does not have to be labelled due to the calculation procedure of international guidelines.
Classification system:
The classification was made according to the latest editions of international substances lists, and is expanded upon by company and technical literature data.

Label elements

Labelling according to EU guidelines:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

(Contd. on page 2)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

Trade name: T4 DNA Ligase

(Contd. of page 1)

Classification system:
NFPA ratings (scale 0 - 4)
Health = 0
Fire = 1
Reactivity = 0
HMIS-ratings (scale 0 - 4)
Health = 0
Fire = 1
Reactivity = 0
OSHA Hazard Overview (Criteria according to 29CFR1910.1200): *Not applicable*
Target Organ(s): *May cause Kidney damage (Nephrotoxin)*

*
4 First aid measures

General information: *No special measures required.*
After inhalation: *Supply fresh air; consult doctor in case of complaints.*
After skin contact: *Generally the product does not irritate the skin.*
After eye contact: *Rinse opened eye for several minutes under running water.*
After swallowing: *If symptoms persist consult doctor.*

*
5 Firefighting measures

Suitable extinguishing agents:
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Special hazards arising from the substance or mixture *None known*
Protective equipment: *No special measures required.*

*
6 Accidental release measures

Personal precautions, protective equipment and emergency procedures *Not required.*
Environmental precautions: *Do not allow to enter sewers/ surface or ground water.*
Methods and material for containment and cleaning up:
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Reference to other sections
No dangerous substances are released.
See Section 7 for information on safe handling.
See Section 13 for disposal information.

*
7 Handling and storage

Handling:
Precautions for safe handling *No special measures required.*
Information about protection against explosions and fires: *No special measures required.*
Storage:
Requirements to be met by storerooms and receptacles: *No special requirements.*
Information about storage in one common storage facility: *Not required.*
Further information about storage conditions: *None.*
Specific end use(s) *No further relevant information available.*

(Contd. on page 3)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: T4 DNA Ligase

(Contd. of page 2)

*
8 Exposure controls/personal protection

Components with limit values that require monitoring at the workplace:	
56-81-5 glycerol	
PEL	15* 5** mg/m³ *total dust **respirable fraction
TLV	10* ppm *Mist

Additional information: The lists that were valid during the creation were used as basis.
Personal protective equipment:
General protective and hygienic measures:
The usual precautionary measures for handling chemicals should be followed.
Breathing equipment: Not required.
Protection of hands:
Protective gloves
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
Material of gloves
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer. As the product is a preparation of several substances, the resistance of the glove material can not be calculated in advance and has therefore to be checked prior to the application.
Eye protection: Goggles recommended during refilling.

9 Physical and chemical properties

General Information	
Appearance:	
Form:	Fluid
Color:	Colorless
Odor:	Characteristic
Odour threshold:	Not determined.
pH-value at 20°C (68 °F):	7.4
Change in condition	
Melting point/Melting range:	Undetermined.
Boiling point/Boiling range:	100°C (212 °F)
Flash point:	> 100°C (> 212 °F)
Flammability (solid, gaseous):	Not applicable.
Ignition temperature:	400°C (752 °F)
Decomposition temperature:	Not determined.
Auto igniting:	Product is not selfigniting.
Danger of explosion:	Product does not present an explosion hazard.
Explosion limits:	
Lower:	0.9 Vol %
Upper:	Not determined.
Vapor pressure at 20°C (68 °F):	0.1 hPa
Density:	Not determined.
Relative density	Not determined.

(Contd. on page 4)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: T4 DNA Ligase

(Contd. of page 3)

Vapour density	Not determined.
Evaporation rate	Not determined.
Solubility in / Miscibility with	
Water:	Not miscible or difficult to mix.
Segregation coefficient (n-octonol/water):	Not determined.
Viscosity:	
Dynamic:	Not determined.
Kinematic:	Not determined.
Solvent content:	
Organic solvents:	50.0 %
Water:	49.2 %
VOC content:	50.0 %
Solids content:	0.6 %
Other information	No further relevant information available.

10 Stability and reactivity

Thermal decomposition / conditions to be avoided: No decomposition if used according to specifications.
Incompatible materials: No further relevant information available.
Hazardous decomposition products: No dangerous decomposition products known.

11 Toxicological information

Acute toxicity:
LD/LC50 values that are relevant for classification: No data available
Primary irritant effect:
on the skin: No irritant effect.
on the eye: Irritating effect.
Sensitization: No sensitizing effects known.
Additional toxicological information:
The product is not subject to classification according to internally approved calculation methods for preparations:
When used and handled according to specifications, the product does not have any harmful effects according to our experience and the information provided to us.

*
12 Ecological information

Aquatic toxicity: Not harmful to the aquatic environment
Persistence and degradability Not available
Behavior in environmental systems:
Bioaccumulative potential Not known
Ecotoxical effects:
Remark: Not available
Additional ecological information:
General notes:
Water hazard class 1 (Self-assessment): slightly hazardous for water

(Contd. on page 5)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: T4 DNA Ligase

(Contd. of page 4)

Do not allow undiluted product or large quantities of it to reach ground water, water course or sewage system.

13 Disposal considerations

Waste treatment methods
Recommendation:
Disposal should be in accordance with applicable regional, national and local laws and regulations.
Refer to Section 7: Handling and Storage and Section 8: Exposure Control/Personal Protection for additional handling information and protection of employees.

Uncleaned packagings:
Recommendation: *Disposal must be made according to official regulations.*

*

14 Transport information

Contact Promega Safety Department for additional transportation information

DOT regulations:	*Not regulated*
Hazard class:	*-*
Land transport ADR/RID (cross-border):	*Not regulated*
ADR/RID class:	*-*
UN-Number:	*Not regulated*
Maritime transport IMDG:	
IMDG Class:	*-*
Marine pollutant:	*No*
Air transport ICAO-TI and IATA-DGR:	*Not regulated*
ICAO/IATA Class:	*-*

*

15 Regulatory information

Sara

Section 355 (extremely hazardous substances):
None of the ingredients are listed.

Section 313 (Specific toxic chemical listings):
None of the ingredients are listed.

TSCA (Toxic Substances Control Act):

56-81-5	*glycerol*
7447-40-7	*potassium chloride*
1185-53-1	*2-Amino-2-(hydroxymethyl)-1,3-propanediolhydrochloride*
7732-18-5	*water, pure*

Proposition 65

Chemicals known to cause cancer:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for females:
None of the ingredients are listed.

(Contd. on page 6)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: T4 DNA Ligase

(Contd. of page 5)

Chemicals known to cause reproductive toxicity for males:
None of the ingredients are listed.

Chemicals known to cause developmental toxicity:
None of the ingredients are listed.

Cancerogenity categories

EPA (Environmental Protection Agency)
None of the ingredients are listed.

IARC (International Agency for Research on Cancer)
None of the ingredients are listed.

NTP (National Toxicology Program)
None of the ingredients are listed.

TLV (Threshold Limit Value established by ACGIH)
None of the ingredients are listed.

MAK (German Maximum Workplace Concentration)
None of the ingredients are listed.

NIOSH-Ca (National Institute for Occupational Safety and Health)
None of the ingredients are listed.

OSHA-Ca (Occupational Safety & Health Administration)
None of the ingredients are listed.

Product related hazard informations:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

National regulations:

Class	Share in %
Wasser	49.2
NK	50.0

Water hazard class: Water hazard class 1 (Self-assessment): slightly hazardous for water.

16 Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Department issuing MSDS:
Promega Corporation
Environmental Health and Safety Department
2800 Woods Hollow Road
Madison, WI
Ph:(608)274-4330
*** Data compared to the previous version altered.**

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 *Reviewed on 03/08/2011*

*
1 Identification of the substance/mixture and of the company/undertaking

Product identifier

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

Article number: *C671*
Application of the substance / the preparation *Laboratory chemicals*

Details of the supplier of the safety data sheet
Manufacturer/Supplier:

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
U.S.A.
1-800-356-9526 or (608)-274-4330

Information department: *MSDS author: Regulatory.Affairs@promega.com*
Emergency telephone number:
For Chemical Emergency ONLY (spill, leak, fire, exposure or accident), call CHEMTREC at 1-800-424-9300
For call originating outside the United States dial 001-703-527-3887

*
2 Composition/information on ingredients

Chemical characterization: Mixtures
Description: *Mixture of the substances listed below with nonhazardous additions.*

Dangerous components:		
25322-68-3	*Polyethlyene Glycol*	*5-10%*

Additional information: *For the wording of the listed risk phrases refer to section 15.*

*
3 Hazards identification

Classification of the substance or mixture

Classification according to Directive 67/548/EEC or Directive 1999/45/EC
Not applicable. Product has been classified as non-hazardous.
Information concerning particular hazards for human and environment:
The product does not have to be labelled due to the calculation procedure of international guidelines.
Classification system:
The classification was made according to the latest editions of international substances lists, and is expanded upon by company and technical literature data.

Label elements

Labelling according to EU guidelines:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

(Contd. on page 2)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

(Contd. of page 1)

Classification system:
NFPA ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
HMIS-ratings (scale 0 - 4)
Health = 0
Fire = 0
Reactivity = 0
OSHA Hazard Overview (Criteria according to 29CFR1910.1200): *Not applicable*
Target Organ(s): *Not applicable or unknown*

*
4 First aid measures

General information: *No special measures required.*
After inhalation: *Supply fresh air; consult doctor in case of complaints.*
After skin contact: *Generally the product does not irritate the skin.*
After eye contact: *Rinse opened eye for several minutes under running water.*
After swallowing: *If symptoms persist consult doctor.*

*
5 Firefighting measures

Suitable extinguishing agents:
CO2, extinguishing powder or water spray. Fight larger fires with water spray or alcohol resistant foam.
Special hazards arising from the substance or mixture *None known*
Protective equipment: *No special measures required.*

*
6 Accidental release measures

Personal precautions, protective equipment and emergency procedures *Not required.*
Environmental precautions: *Do not allow to enter sewers/ surface or ground water.*
Methods and material for containment and cleaning up:
Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust).
Reference to other sections
No dangerous substances are released.
See Section 7 for information on safe handling.
See Section 13 for disposal information.

*
7 Handling and storage

Handling:
Precautions for safe handling *No special measures required.*
Information about protection against explosions and fires: *The product is not flammable.*
Storage:
Requirements to be met by storerooms and receptacles: *No special requirements.*
Information about storage in one common storage facility: *Not required.*
Further information about storage conditions: *None.*
Specific end use(s) *No further relevant information available.*

(Contd. on page 3)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

(Contd. of page 2)

*
8 Exposure controls/personal protection

Components with limit values that require monitoring at the workplace:

25322-68-3 Polyethlyene Glycol

WEEL	10 mg/m³

Additional information: The lists that were valid during the creation were used as basis.
Personal protective equipment:
General protective and hygienic measures:
The usual precautionary measures for handling chemicals should be followed.
Breathing equipment: Not required.
Protection of hands:
Protective gloves
Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation
Material of gloves
The selection of the suitable gloves does not only depend on the material, but also on further marks of quality and varies from manufacturer to manufacturer. As the product is a preparation of several substances, the resistance of the glove material can not be calculated in advance and has therefore to be checked prior to the application.
Eye protection: Goggles recommended during refilling.

9 Physical and chemical properties

General Information	
Appearance:	
Form:	Fluid
Color:	Colorless
Odor:	Characteristic
Odour threshold:	Not determined.
pH-value at 20°C (68 °F):	7.8
Change in condition	
Melting point/Melting range:	Undetermined.
Boiling point/Boiling range:	100°C (212 °F)
Flash point:	Not applicable.
Flammability (solid, gaseous):	Not applicable.
Ignition temperature:	
Decomposition temperature:	Not determined.
Auto igniting:	Product is not selfigniting.
Danger of explosion:	Product does not present an explosion hazard.
Explosion limits:	
Lower:	Not determined.
Upper:	Not determined.
Vapor pressure:	Not determined.
Density:	Not determined.
Relative density	Not determined.
Vapour density	Not determined.
Evaporation rate	Not determined.

(Contd. on page 4)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

(Contd. of page 3)

Solubility in / Miscibility with	
Water:	Not miscible or difficult to mix.
Segregation coefficient (n-octonol/water):	Not determined.
Viscosity:	
Dynamic:	Not determined.
Kinematic:	Not determined.
Solvent content:	
Organic solvents:	0.0 %
Water:	88.2 %
Other information	No further relevant information available.

10 Stability and reactivity

Thermal decomposition / conditions to be avoided: No decomposition if used according to specifications.
Incompatible materials: No further relevant information available.
Hazardous decomposition products: No dangerous decomposition products known.

11 Toxicological information

Acute toxicity:
LD/LC50 values that are relevant for classification: No data available
Primary irritant effect:
on the skin: No irritant effect.
on the eye: Irritating effect.
Sensitization: No sensitizing effects known.
Additional toxicological information:
The product is not subject to classification according to internally approved calculation methods for preparations:
When used and handled according to specifications, the product does not have any harmful effects according to our experience and the information provided to us.

*
12 Ecological information

Aquatic toxicity: Not harmful to the aquatic environment
Persistence and degradability Not available
Behavior in environmental systems:
Bioaccumulative potential Not known
Ecotoxical effects:
Remark: Not available
Additional ecological information:
General notes:
Water hazard class 1 (Self-assessment): slightly hazardous for water
Do not allow undiluted product or large quantities of it to reach ground water, water course or sewage system.

(Contd. on page 5)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

(Contd. of page 4)

*
13 Disposal considerations

Waste treatment methods
Recommendation:
Disposal should be in accordance with applicable regional, national and local laws and regulations.
Refer to Section 7: Handling and Storage and Section 8: Exposure Control/Personal Protection for additional handling information and protection of employees.

Uncleaned packagings:
Recommendation: Disposal must be made according to official regulations.

*
14 Transport information

Contact Promega Safety Department for additional transportation information

DOT regulations:	Not regulated
Hazard class:	-
Land transport ADR/RID (cross-border):	Not regulated
ADR/RID class:	-
UN-Number:	Not regulated
Maritime transport IMDG:	
IMDG Class:	-
Marine pollutant:	No
Air transport ICAO-TI and IATA-DGR:	Not regulated
ICAO/IATA Class:	-

*
15 Regulatory information

Sara

Section 355 (extremely hazardous substances):
None of the ingredients are listed.

Section 313 (Specific toxic chemical listings):
None of the ingredients are listed.

TSCA (Toxic Substances Control Act):

25322-68-3	Polyethlyene Glycol
1185-53-1	2-Amino-2-(hydroxymethyl)-1,3-propanediolhydrochloride
7786-30-3	Magnesium Chloride
3483-12-3	DL-Dithiothreitol
7732-18-5	water, pure

Proposition 65

Chemicals known to cause cancer:
None of the ingredients are listed.

Chemicals known to cause reproductive toxicity for females:
None of the ingredients are listed.

(Contd. on page 6)

Material Safety Data Sheet
acc. to ISO/DIS 11014

Printing date 03/08/2011 Reviewed on 03/08/2011

Trade name: 2X Rapid Ligation Buffer, T4 DNA Ligase

(Contd. of page 5)

Chemicals known to cause reproductive toxicity for males:
None of the ingredients are listed.

Chemicals known to cause developmental toxicity:
None of the ingredients are listed.

Cancerogenity categories

EPA (Environmental Protection Agency)
None of the ingredients are listed.

IARC (International Agency for Research on Cancer)
None of the ingredients are listed.

NTP (National Toxicology Program)
None of the ingredients are listed.

TLV (Threshold Limit Value established by ACGIH)
None of the ingredients are listed.

MAK (German Maximum Workplace Concentration)
None of the ingredients are listed.

NIOSH-Ca (National Institute for Occupational Safety and Health)
None of the ingredients are listed.

OSHA-Ca (Occupational Safety & Health Administration)
None of the ingredients are listed.

Product related hazard informations:
Observe the general safety regulations when handling chemicals.
The product is not subject to identification regulations according to directives on hazardous materials.

National regulations:

Water hazard class: Water hazard class 1 (Self-assessment): slightly hazardous for water.

*
16 Other information

This information is based on our present knowledge. However, this shall not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship.

Department issuing MSDS:
Promega Corporation
Environmental Health and Safety Department
2800 Woods Hollow Road
Madison, WI
Ph:(608)274-4330
*** Data compared to the previous version altered.**

Map of recombinant human Ad-Cre-GFP (cat#1700)

I: Human Ad5 sequences (wt 1-458; includes 5' L-ITR and packaging signal)

II: CMV-Cre-polyA-CMV-GFP-polyA

III: Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR
E3 deletion: nts 28587 - 30464)

Map of recombinant human Ad-CMV-GFP

I: Human Ad5 sequences (wt 1-458; includes 5' L-ITR and packaging signal)

II: CMV-GFP-PolyA

III: Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR)

User Manual

Adeno-X™ Maxi Purification Kit User Manual

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Cat. Nos. 631532
631533
PT3680-1 (PR7X2385)
Published 15 October 2007

Table of Contents

I. Introduction & Protocol Overview 3

II. List of Components 5

III. Additional Materials Required 5

IV. Safety & Handling of Adenoviruses 6

V. Adenovirus Purification Protocol 7

A. Test the Titer of the Adenoviral Stock 7

B. Amplify Adenovirus in HEK 293 Cells 7

C. Harvest Adenovirus 9

D. Purify Adenovirus 9

VI. References 11

List of Figures

Figure 1. Principle of Adeno-X Virus Purification 4

Figure 2. The Adeno-X Virus Purification Assembly 4

Figure 3. Overview of the Adeno-X Maxi Purification Protocol 8

Note: The viral supernatants produced by transfecting HEK 293 cells with recombinant Adeno-X Viral DNA could, depending on your DNA insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant adenovirus. **The user is strongly advised not to create adenoviruses capable of expressing known oncogenes**.

Appropriate NIH, regional, and institutional guidelines apply, as well as guidelines specific to other countries. NIH guidelines require that adenoviral production and transduction be performed in a Biosafety Level 2 facility. For more information, see appropriate HHS publications. A link to the *NIH Guide to Biosafety in Microbiological and Biomedical Laboratories* can be found at **www.clontech.com/clontech/expression/adeno**. Section IV in this User Manual contains a brief description of Biosafety Level 2 as well as other general information and precautions.

I. Introduction & Protocol Overview

The **Adeno-X Maxi Purification Kit** is a complete chromatography-based system for purifying and concentrating recombinant adenovirus. It provides a superior alternative to cesium chloride (CsCl) density gradient centrifugation. Although centrifugation in CsCl is an extremely effective method for purifying adenovirus, it is also time-consuming, technically demanding, and toxic (Graham & Prevec, 1991). Furthermore, the procedure is restrictive in that it is not easily scaled up or down. The Adeno-X Maxi Purification Kit, on the other hand, can be scaled up or down without difficulty. The kit also allows you to purify a maximal amount of recombinant adenovirus directly from the cell pellet. You simply wait until the cytopathic effect (CPE) is complete—when the viral titer is highest—harvest the cells and purify the virus. The Adeno-X Purification Kit is not only faster than CsCl methods, it is also easier, safer, and just as effective.

A chromatographic method

The Adeno-X Maxi Purification Kit lets you purify adenovirus chromatographically, using an adsorbent filter membrane that selectively binds adenoviral particles based on their distinctive surface-associated properties (Figure 1). The membrane is housed in a small, single-use cartridge that fits securely on a disposable Luer-Lok™ syringe. For added convenience, the syringe-filter assembly comes completely pre-assembled and ready to use (Figure 2). This apparatus includes a one-way valve that makes it easier to load and wash the filter. The virus-containing medium is simply drawn into the syringe through the one-way valve and pushed through the purification filter cartridge. Here, the adenoviral particles are trapped and effectively removed from the solution. Once bound, the viral particles can be eluted with a small volume of 1X Elution Buffer (provided).

The purification protocol

The procedure is simple. After the cytopathic effect is complete, the cells are harvested, lysed, and centrifuged. The resulting supernatant is then treated with Benzonase® Nuclease to digest nucleic acids, and cleared (clarified) through a syringe-tip pre-filter. Next, a syringe is used to push the virus-containing extract through the purification cartridge, where the adenoviral particles bind to the filter. The bound particles are then eluted with a small volume of buffer. The entire protocol, from harvest to purification, takes just 1-1.5 hours to complete (Figure 3). Aliquots of the purified, high-titer adenoviral stock can be stored in Elution Buffer at –70°C. Please note: All of these steps must be conducted under sterile conditions in a Biosafety Level 2 certified hood.

I. Introduction & Protocol Overview *continued*

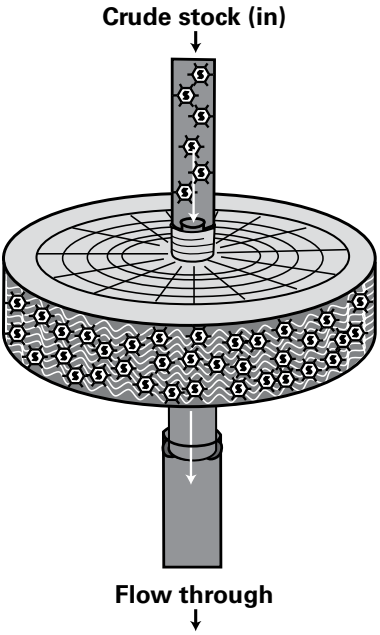


Figure 1. Principle of Adeno-X virus purification. The Adeno-X Purification Filter consists of several layers that selectively bind adenoviral particles as they pass through. The filter is enclosed in a small cylindrical cartridge that resembles a radial flow column, complete with entrance and exit ports. The Maxi purification cartridge is 3.5 cm wide and 1 cm thick.

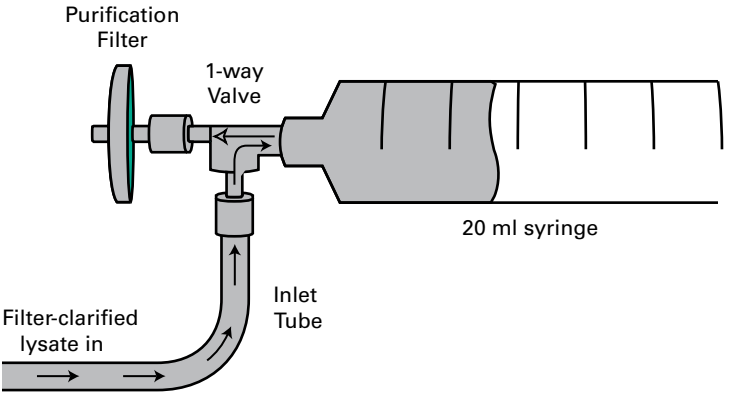


Figure 2. The Adeno-X Maxi Purification Assembly. The apparatus comes pre-assembled.

II. List of Components

Store Benzonase® Nuclease at –20°C. Store all other components at room temperature.

The **Adeno-X Maxi Purification Kits** (Cat. Nos. 631532 & 631533) contain sufficient reagents for 2 and 6 Maxi purifications, respectively.

	Cat. No. 631532	Cat. No. 631533	
•	15 ml	2x15 ml	1X Equilibration Buffer
•	15 ml	2x15 ml	1X Dilution Buffer
•	60 ml	2x60 ml	1X Wash Buffer
•	12 ml	2x12 ml	1X Elution Buffer
•	40 µl	40 µl	Benzonase Nuclease (25 U/µl)
•	2	6	Syringe-tip Pre-filter
•	2	6	Adeno-X Maxi Purification Assembly
•	2	6	Syringe (5 ml)
•	2	6	Syringe (20 ml)

III. Additional Materials Required

The following materials are required but not supplied.

- **1X Formulation Buffer**
  2.5% glycerol (w/v), 25 mM NaCl, and 20 mM Tris-HCl, pH 8.0 (GTS buffer; Hoganson, *et al.,* 2002).
- **Tissue culture plates and flasks**
  (e.g. 10 or 15 cm plates, T75 or T175 flasks, or roller bottles)
- **Centrifuge**
  (Swinging-bucket and fixed-angle rotors compatible with 15 ml, 50 ml, and if needed, 100 ml centrifuge tubes)
- **Sterile 50 ml centrifuge tubes**

IV. Safety & Handling of Adenoviruses

The protocols in this User Manual require the production, handling, and storage of infectious adenovirus. It is imperative to fully understand the potential hazards of and necessary precautions for the laboratory use of adenoviruses.

The National Institute of Health and Center for Disease Control have designated adenoviruses as Level 2 biological agents.This distinction requires the maintenance of a Biosafety Level 2 facility for work involving this virus and others like it.The viruses packaged by transfecting HEK 293 cells with the adenoviral-based vectors described here are capable of infecting human cells.These viral supernatants could, depending on your gene insert, contain potentially hazardous recombinant virus. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*.

For these reasons, due caution must be exercised in the production and handling of any recombinant adenovirus. **The user is strongly advised not to create adenoviruses capable of expressing known oncogenes.**

For more information on Biosafety Level 2, see the following reference:

- *Biosafety in Microbiological and Biomedical Laboratories*, 4th Edition (May 1999) U.S. Department of Health and Human Services. (Available at http://bmbl.od.nih.gov.)

Biosafety Level 2:
The following information is a brief description of Biosafety Level 2. *It is neither detailed nor complete*. Details of the practices, safety equipment, and facilities required for Biosafety Level 2 are available in the above publication. If possible, observe and learn the practices described below from someone who has experience working with adenoviruses.

- Practices:
  - perform work in a limited access area
  - post biohazard warning signs
  - avoid generating aerosols
  - decontaminate potentially infectious wastes before disposal
  - take precautions with sharps (e.g., syringes, blades)
- Safety equipment:
  - biological safety cabinet, preferably Class II (i.e., a laminar flow hood with microfilter [HEPA filter] that prevents release of aerosols)
  - protective laboratory coats, face protection, double gloves
- Facilities:
  - autoclave for decontamination of wastes
  - unrecirculated exhaust air
  - chemical disinfectants available for spills

V. Adenovirus Purification Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

A. Test the Titer of the Adenoviral Stock

1. Determine the optimal amount of viral stock needed to infect your cells. To do this, test the viral titer in a small scale cytopathic effect assay, using culture conditions that closely approximate those that will be used for the actual adenoviral prep.
   a. Seed low passage HEK 293 cells on a 12-well tissue culture dish at a density of $1x10^5$ cells/cm^2, using 0.28 ml/cm^2 medium. At 1 ml/well, this is equivalent to ~$3.57x10^5$ cells/well.
   b. Place the cells at 37°C and 5% CO_2 while you dilute the adenovirus.
   c. Infect the cells with a range of adenoviral concentrations. If you know the titer of your stock, aim for a multiplicity of infection (M.O.I.) of 1-2. If you don't know the titer, infect the cells with a range of dilutions, starting with 1 µl of stock and serially diluting 3X from there.
   d. Incubate the cells at 37°C and 5% CO_2 until the cytopathic effect is complete; this should take approximately 3-5 days.

   Note: The cytopathic effect (CPE) refers to the morphological changes that the cells undergo after infection. Infected cells typically remain intact, but round up and may detach from the dish individually or in "grape-like" clusters that float in the medium. **Optimally, the purification assay should be performed when 50% of the cells are detached, and the remainder are attached but rounded.**
2. Use the results from the test titer to determine the optimal amount of virus to use in the actual purification prep. The full scale prep requires seeding several 150 mm cell culture dishes with $1.46x10^7$ cells/dish. Therefore, if 0.1 µl of adenoviral stock optimally infected the $3.57x10^5$ cells used to seed the test titer, you would need 4 µl of virus for each 150 mm dish in the actual prep.

B. Amplify Adenovirus in HEK 293 Cells

1. Detach low passage HEK 293 cells with trypsin, wash, and count.
2. In a 50 ml conical tube, dilute the cells to a final density of $1.83x10^6$ cells/ml in a total of 40 ml.
3. Add virus to the cells and mix.

   Note: Perform the test titer in Part A to determine the optimal amount of virus to add in this step.
4. Aliquot 33 ml of fresh medium onto each of five 150 mm cell culture dishes.
5 Aliquot 8 ml of virus-infected cells (from step 3) onto each of the five 150 mm cell culture dishes; make sure the cells are evenly distributed.

   Note: This is equivalent to $1.46x10^7$ cells/plate.

V. Adenovirus Purification Protocol *continued*

6. Incubate the cells at 37°C and 5% CO_2 until the CPE is complete; this should take approximately 3-5 days.

   Note: Optimally, the purification assay should be performed when 50% of the cells are detached, and the remainder are attached but rounded.

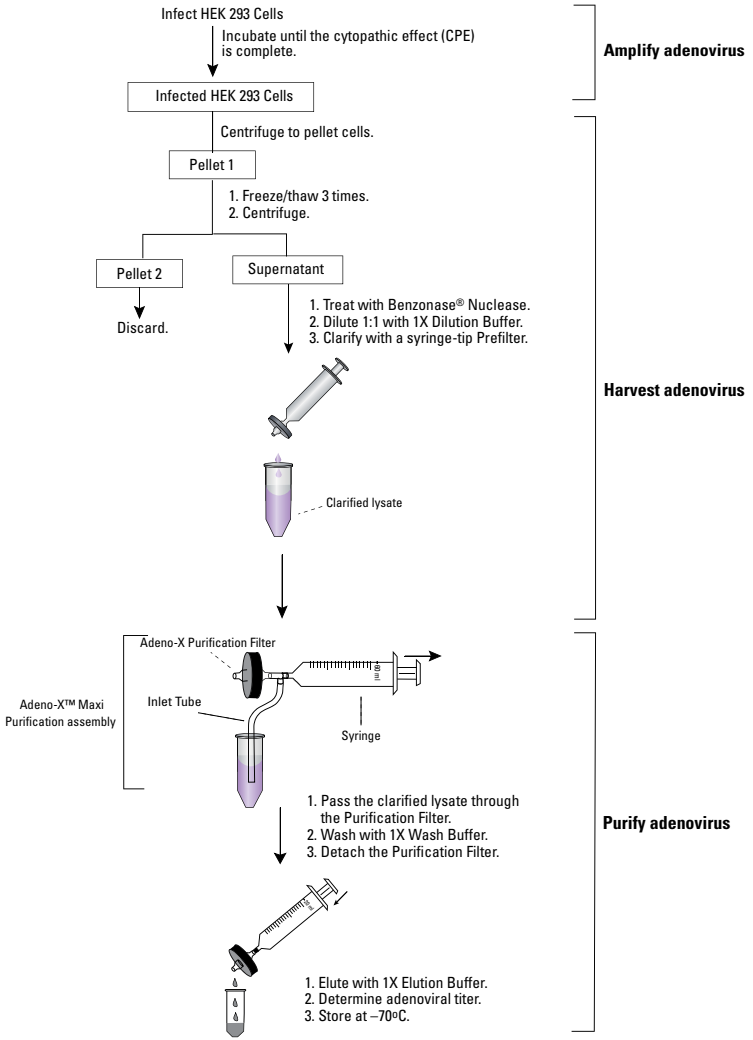


Figure 3. Overview of the Adeno-X Maxi Purification Protocol.

V. Adenovirus Purification Protocol *continued*

C. Harvest Adenovirus (See Figure 3 for a procedural diagram)

1. Pellet the cells by centrifugation in a swinging-bucket centrifuge at 1,500 rpm (500 x g for Table-Top Beckman Centrifuge GH3.8 rotor) for 10 min.
2. Discard the supernatant.
3. Resuspend the pellet in 5 ml of fresh medium.

   Attention: Do not use PBS, as it inhibits some endonucleases. Endonuclease is used below to remove contaminating cellular nucleic acids from filter-clarified cell lysate.
4. Lyse the cells with three consecutive freeze-thaw cycles; briefly freeze the cells in a dry ice/ethanol bath, then thaw them in a 37°C water bath.
5. After thawing for the final time, centrifuge the lysate at 3,000 rpm (~1,500 x g for Table-Top Beckman Centrifuge GH3.8 rotor) for 5 min to pellet the debris.
6. Collect and save the supernatant in a sterile centrifuge tube. Discard the pellet.
7. Add 5 µl Benzonase® Nuclease and incubate for 30 min at 37° C.

   Note: Nuclease treatment decreases the viscosity of the solution so that it can be drawn more easily through the Adeno-X Purification Filter.
8. Measure the volume of the sample with a pipet, then add an equal volume of 1X Dilution Buffer (approximately 5 ml).

   Note: The solution may turn slightly purple if you resuspended the original cell pellet in fresh medium (step 3).
9. Clarify the lysate by filtering it through the 0.45 µm Syringe-tip Pre-filter, using a 20 ml syringe (provided).

D. Purify Adenovirus

1. Equilibrate the Filter Assembly with 5 ml 1X Equilibration Buffer. To do this, place the inlet tube into the buffer and draw the buffer into the syringe by pulling back on the plunger. Press lightly on the plunger to push the buffer through the Filter at a flow rate of approximately 3 ml/min (this is approximately 1 drop/sec).

   Note: Do not force residual air through the Filter. To remove air from the syringe, disconnect the Filter, force the air out of the syringe with the plunger, then reposition the Filter on the syringe.
2. Place the inlet tube into the clarified lysate, and draw the lysate into the syringe.
3. Load the adenovirus onto the Purification Filter. This is done by pushing the lysate through the Filter at a rate of ~3 ml/min to allow the virus to bind.
4. Transfer the inlet tube to a sterile tube containing 20 ml 1X

V. Adenovirus Purification Protocol *continued*

Wash Buffer. Push the Wash Buffer through the filter at a rate of ~3 ml/min

5. Remove the Filter from the Assembly.
6. To elute the adenovirus, attach the Filter to a new 5 ml syringe containing 3 ml 1X Elution Buffer. Push 1 ml of Elution Buffer through the filter into a sterile 15 ml conical tube. Incubate the Filter at room temperature for 5 min, then push the remaining elution buffer through. Use residual air in the syringe to push any remaining virus through the Filter. Combine the eluate to obtain approximately 3 ml total.
7. Determine the adenoviral titer.

   Notes:

   - We recommend using the Adeno-X Rapid Titer Kit (Cat. No. 631028).
   - For information on how to measure the adenoviral particle concentration by its optical density at 260nm (OD_{260} Assay), please go to **www.clontech.com/support/titer**
8. The adenovirus can be used immediately, or aliquoted and stored at –70°C.

Note:

For improved long-term stability, and proper tonicity for *in vivo* applications, we recommend a buffer exchange of the eluted adenovirus into 1X Formulation Buffer.

1X Formulation Buffer:
2.5% glycerol (w/v), 25 mM NaCl, and 20 mM Tris-HCl, pH 8.0 (GTS buffer; Hoganson, *et al.,* 2002)

VI. References

Adeno-X Virus Purification Kits (July 2002) *Clontechniques* **XVIII**(3):10–11.

Adeno-X Rapid Titer Kit (April 2002) *Clontechniques* **XVII** (2):16–17.

Graham, F. L. & Prevec, L. (1991) Manipulation of adenovirus vectors. In *Methods in Molecular Biology, Vol. 7: Gene Transfer and Expression Protocols.* Ed. Murray, E. J. (Human Press Inc., Clifton, NJ), pp. 109–128.

Hoganson, D. K., Ma, J. C., Asato, L., Ong, M., Printz, M. A., Huyghe, B. G., Sosnowski, B . A. & D'Andrea, M. J. (2002) Development of a stable adenoviral vector formulation. *Bioprocessing J.* **1**(1):43–48.

Hutchins, B. (2002) Development of a reference material for characterizing adenovirus vectors. *Bioprocessing J.* **1**(1):25–28.

Notes

Notice to Purchaser

Clontech products are to be used for research purposes only.They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

Benzonase® is a registered trademark of Merck KGaA,

Luer-Lok™ is a trademark of - Becton, Dickinson and Company

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

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 fever, 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

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Material Safety Data Sheet

SECTION 1. PRODUCT IDENTIFICATION

Catalog Number: RTV-xxx
Product Name: Retroviral Expression Vectors

MANUFACTURER:

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126

EMERGENCY CONTACT:

+1 858 271 6500
info@cellbiolabs.com

SECTION 2. COMPOSITION/INFORMATION ON INGREDIENTS

Plasmid DNA in TE Buffer or Bacterial Glycerol Stock

SECTION 3. WASTE DISPOSAL

For small quantities: Cautiously add to a large stirred excess of water. Adjust the pH to neutral. Flush the aqueous solutions down the drain with plenty of water.

SECTION 4. FIRST-AID MEASURES

- IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
- IN CASE OF SKIN CONTACT, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. REMOVE CONTAMINATED CLOTHING AND SHOES. CALL A PHYSICIAN.
- IN CASE OF CONTACT WITH EYES, FLUSH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. ASSURE ADEQUATE FLUSHING BY SEPARATING THE EYELIDS WITH FINGERS. CALL A PHYSICIAN.

SECTION 5. SAFETY HANDLING PROCEDURES

- Should be handled by trained personnel observing good laboratory practices.
- Avoid breathing vapor.
- Avoid skin contact or swallowing.
- May cause allergic reaction in sensitized individuals.

SECTION 6. ACCIDENTAL RELEASE MEASURES

EVACUATE AREA. WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES. ABSORB WITH SAND OR VERMICULITE, SWEEP UP, PLACE IN A BAG AND HOLD FOR WASTE DISPOSAL. AVOID RAISING DUST. VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

The above information is believed to be correct but does not purport to be all inclusive and should be used only as a guide for experienced personnel. Cell Biolabs, Inc. shall not be held liable for any damage resulting from the handling or from contact with the above product(s).

7758 Arjons Drive, San Diego, CA 92126
Phone (858) 271 6500
US Toll Free (888) CBL 0505
Fax (858) 271 6514
info@cellbiolabs.com

pBABE-Puro Retroviral Vector

CATALOG NUMBER: RTV-001-puro **STORAGE:** -20ºC

QUANTITY AND CONCENTRATION: 10 µg at 0.5 µg/µL in TE

Background

Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. Cell Biolabs' retrovirus vector is based on the pBABE vector system, which is derived from Moloney murine leukemia virus (MMLV). The vector provides the viral package signal, transcription and processing elements, and a target gene. The viral *env* gene, produced by the package cell line, encodes the envelop protein, which determines the viral infectivity range. Transfection into a package cell line produces high-titer, replication-incompetent viruses. In addition to transfer and expression of exogenous genes in mammalian cells, recently, retroviruses are used to express silencing RNAs (siRNA) to decrease the expression of target genes both *in vitro* and *in vivo*.

The vector contains the bacterial origin of replication, ampicillin-resistance gene, and puromycin-resistance gene for the grwoth of infected mammalian cells to select stable cell lines (Figure 1).

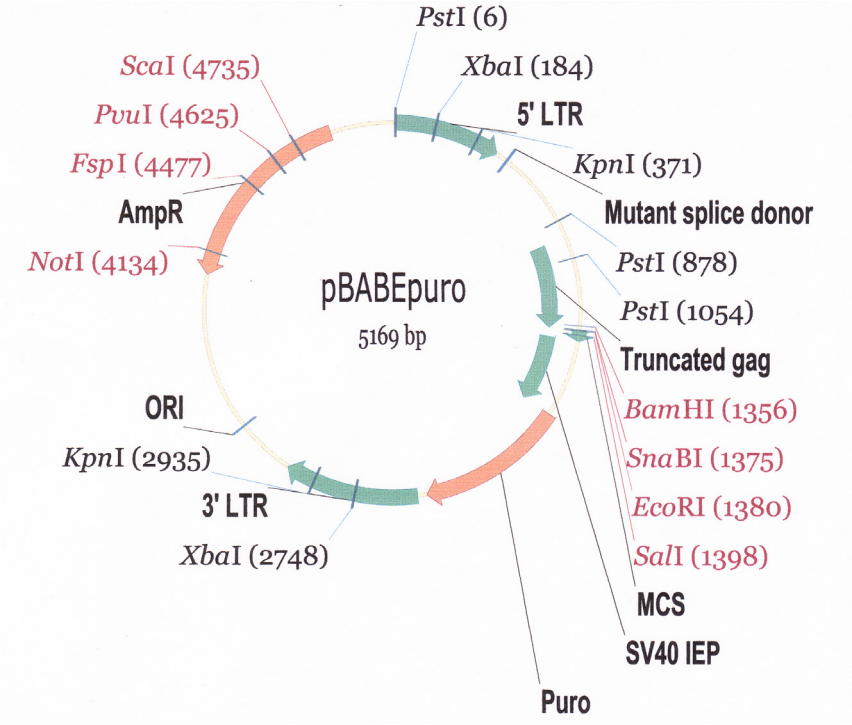


Figure 1. pBABE-Puro Retroviral Vector Map

Safety Consideration

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

References

1. Morgenstern, J. P. and H Land. (1990) *Nuc. Acid Res.* 18, 3587-3596.
2. Coffin, J. M. and H. E. Varmus, *Retroviruses*, Cold Spring Harbor Press, NY.
3. Schuck S, Manninen A, Honsho M, Fullekrug J and Simons K. (2004) *Proc Natl Acad Sci U S A.* 101, 4912-4917.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS*'s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

pSUPER RNAi System™

VECTOR: pSUPER.retro.neo+GFP
CATALOG#: VEC-PRT-0005/0006

Length: 7641 bp

Key Sites

BglII: 2424
HindIII: 1441
EcoRI: 2645
SalI: 1426
XhoI: 1420

Vector Features

PGK promoter: 2770-3168
EGFP ORF: 3186-3919
Neo ORF: 3926-4895
H1 promoter: 2430-2650
Ampicillin resistance ORF: 7443-6577
3' delta LTR: 4910-5277
5' LTR: 8369-513 (homologous to other MSCV LTR)
Stuffer Sequence: 1447-2423

Sequencing primer 5'-GGAAGCCTTGGCTTTTG-3' binding site: 1241-1257
Sequencing primer 5'-GATGACGTCAGCGTTCG-3' binding site: 2645-2629