**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOLOGICAL AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: October 14, 2010**
**Biosafety Website: www.uwo.ca/humanresources/biosafety/**

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

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

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

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

| | |
|---|---|
| PRINCIPAL INVESTIGATOR | S. Jeffrey Dixon |
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| ADDRESS | Dental Sciences Building, Rm 0075 |
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Location of experimental work to be carried out: Building(s): Dental Sciences_ Room(s): 0078, 0078a____

*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_____
GRANT TITLE(S): Ion transport and signaling in skeletal cells: P2 nucleotide receptor function in bone _

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

| Name | UWO E-mail Address | Date of Biosafety Training |
|---|---|---|
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| Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed. | | | | |
|---|---|---|---|---|
| **Biohazard/Biological Agent** | | **How is it Stored?** | **How is it Used?** | **How is it Disposed of?** |
| Microorganisms | Escherichia Coli | In freezer (-80) for long term storage or in Incubator when in use. This applies to agents listed below | Kept in culture for carrying out molecular biology | Bleached. Materials that come in contact are autoclaved |
| | Recombinant Adenovirus - Non replicating | | Cells are infected with modified adenovirus for expression of selected proteins | |
| | | | The following molecular biology reagents are used for expression of various markers in cells | |
| Vectors/ Plasmids | pcDNA3 | | | |
| | pEGFP | | | |
| | PEYFP | | | |
| | pAD/CMV/V5-DEST | | | |
| | pEGFP-LC3 | | | |
| | pCEP4YPet-MAMM YPET | | | |
| | | | | |
| Cells | Human (established): HEK 293 | All cell lines are maintained in liquid nitrogen, with aliquots thawed for expansion in culture | | Bleached. Materials that come in contact are autoclaved |
| | Rodent (established): RAW 264.7, MC3T3-E1, UMR-106, ROS17/2.8 | | | |
| | CHO | | | |
| | Non-human primate (established): COS | | | |
| | Rodent (primary) | Primary cells are prepared freshly and may be maintained in short-term culture | | |
| | | | | |
| Toxins | Pertussis Toxin | Freezer | Is prepared as stock solution then added to cell culture dishes for short-term treatment for blocking cell signaling by certain receptors. | Is used in extremely low volumes and concentrations. The remaining solutions are diluted and are then bleached. |
| | | | | |

**Please include a one page research summary or teaching protocol.**

**Ion transport and signaling in skeletal cells: P2 nucleotide receptor function in bone**

**Background**: Studies by us and others have shown that extracellular nucleotides (such as ATP) interact with mammalian and human bone cells through multiple subtypes of P2 cell-surface nucleotide receptors. P2X receptor family members are ligand-gated cation channels; whereas, P2Y family members are G protein-coupled receptors. Since nucleotides are released from cells in response to mechanical stimuli and trauma, they may serve as autocrine/paracrine regulators during mechanotransduction and wound healing in bone. In collaboration with Pfizer, we reported a unique skeletal phenotype in the P2X7 receptor knockout mouse – diminished periosteal bone formation and excessive trabecular bone resorption. Our mechanistic studies revealed that P2X7 receptors enhance osteoblast differentiation in a cell-autonomous manner, in part through production of the potent lipid mediator lysophosphatidic acid. Interestingly, prevalent loss-of-function polymorphisms in the human P2X7 receptor gene (P2RX7) have recently been associated with accelerated bone loss and increased fracture risk in postmenopausal women. However, critical questions remain regarding P2X7 signaling and function in bone. Our overall hypothesis is that P2X7 receptors regulate skeletal remodeling through direct effects on osteoblasts and osteoclasts, and indirectly through a novel mechanism of intercellular signaling in bone.

The following **specific hypotheses** will be tested:

1) Stimulation of osteogenesis by P2X7 requires activation of the transcription factor NFATc1 in osteoblasts.

2) Nucleotides, acting through P2X7 receptors on osteoblasts, induce the rapid release of membrane vesicles that contain bioactive molecules capable of regulating osteoblast and osteoclast activity.

3) Activation of P2X7 receptors on osteoclasts suppresses bone resorption via disruption of the actin cytoskeleton and induction of apoptosis.

4) Skeletal healing and mechanotransduction are impaired in mice lacking the P2X7 receptor.

**Research plan**: We will use calvarial cells and osteoclasts from neonatal rats, P2rx7-/- and wild-type mice. P2X7 receptor activity and function will be assessed by patch clamp and calcium fluorescence. Nuclear translocation of NFATc1 in response to P2X7 agonists will be monitored in real-time by imaging of osteoblasts expressing NFAT-EGFP fusion proteins, permitting unique insights into signaling. Effects of NFATc1 loss-of-function will be assessed by quantifying expression of marker genes using real-time PCR, and osteogenesis using the bone nodule formation assay. A novel mechanism for intercellular communication in bone – P2X7-induced vesicle shedding from osteoblasts – will be characterized by time-lapse, confocal and electron microscopy, and electrophysiology. Released bioactive compounds will be identified by mass spectrometry and immunodetection, and their roles assessed using complementary in vitro and in vivo assays. The effects of nucleotides on osteoclast motility and cytoskeletal dynamics will be studied using live-cell imaging of cells expressing actin-EGFP. Survival and apoptosis will be monitored using light and fluorescence microscopy, and confirmed by TUNEL. Resorptive activity will be quantified using pit formation assays. Skeletal healing and mechanotransduction will be examined in wild-type and P2rx7-/- mice.

**Significance**: Nucleotides may regulate bone remodeling by stimulating P2X7 on osteoblasts and osteoclasts during mechanotransduction and early stages of wound healing. Elucidation of downstream signaling pathways and functions of P2X7 will add to our understanding of osteopenia and impaired skeletal healing in patients with common loss-of-function P2RX7 polymorphisms. P2X7 receptors represent an attractive therapeutic target for inhibiting osteoclast activity while simultaneously promoting bone formation by osteoblasts.

## 1.0 Microorganisms

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

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

| Name of Biological agent(s)* | Is it known to be a human pathogen? YES/NO | Is it known to be an animal pathogen? YES/NO | Is it known to be a zoonotic agent? YES/NO | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|---|---|---|---|---|---|---|
| Escherichia Coli (TOP 10 competent cells-Invitrogen) | x Yes<br>O No | x Yes<br>O No | x Yes<br>O No | 1.5 | | x 1 O 2<br>O 2+ O 3 |
| Recombinant Adenovirus - Non replicating | O Yes<br>x No | O Yes<br>x No | O Yes<br>x No | 0.15 | Invitrogen | O 1 x 2<br>O 2+ O 3 |
| | O Yes<br>O No | O Yes<br>O No | O Yes<br>O No | | | O 1 O 2<br>O 2+ O 3 |
| | O Yes<br>O No | O Yes<br>O No | O Yes<br>O No | | | O 1 O 2<br>O 2+ O 3 |

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

## 2.0 Cell Culture

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

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

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|---|---|---|---|
| Human | O Yes x No | | Not applicable |
| Rodent | x Yes O No | Rats, Mice, Rabbit | 2008-043-06 |
| Non-human primate | O Yes x No | | |
| Other (specify) | O Yes x No | | |

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

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Supplier / Source |
|---|---|---|---|
| Human | x Yes O No | HEK 293 | Invitrogen, ATCC |
| Rodent | x Yes O No | RAW 264.7, CHO, MC3T3-E1, UMR-106, ROS17/2.8 | ATCC |
| Non-human primate | x Yes O No | COS-1 | ATCC |
| Other (specify) | O Yes x No | | |

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

2.3 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 x 2 O 2+ O 3

**3.0 Use of Human Source Materials**

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

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

| **Human Source Material** | **Source/Supplier /Company Name** | **Is Human Source Material Infected With An Infectious Agent? YES/NO** | **Name of Infectious Agent (If applicable)** | **PHAC or CFIA Containment Level (Select one)** |
|---|---|---|---|---|
| Human Blood (whole) or other Body Fluid | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Blood (fraction) or other Body Fluid | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Organs or Tissues (unpreserved) | | O Yes<br>O Unknown | | O 1 O 2<br>O 2+ O 3 |
| Human Organs or Tissues (preserved) | | Not Applicable | | Not Applicable |

**4.0 Genetically Modified Organisms and Cell lines**

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

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

| Bacteria Used for Cloning * | Plasmid(s) ** | Source of Plasmid | Gene Transfected | Describe the change that results from transformation or transfection |
|---|---|---|---|---|
| *Escherichia Coli* (TOP 10 competent cells-Invitrogen) | pcDNA3<br>pEGFP<br>PEYFP<br><br>pEGFP-LC3<br>pCEP4YPet-MAMM YPET | addgene<br>addgene<br>addgene<br><br>addgene<br>addgene | eGFP<br>eGFP<br>eYFP (GFP variant)<br><br>MAP1LC3B, LC3B<br>YPet | These plasmids are all used to express and produce large amounts of the protein of interest in the transfected cells |

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

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

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | Describe the change that results from transduction |
|---|---|---|---|---|
| human adenovirus type 5 (Ad5) | pAd/CMV/V5-DEST | Invitrogen, Cat# V493-20 | human beta actin-EGFP fusion; EGFP | Transient expression of human beta actin-EGFP fusion and EGFP proteins that can be visualized. Cells are appropriately destroyed after every experiment. |

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? x YES O NO

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

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

**5.0 Human Gene Therapy Trials**

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

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

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

5.3 How will the biological agent be administered? _____

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

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

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

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

_____

_____

**7.0 Use of Animal species with Zoonotic Hazards**

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES x No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

**8.0 Biological Toxins**

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

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

8.3 What is the $LD_{50}$ (specify species) of the toxin__18 ug/kg_____

8.4 How much of the toxin is handled at one time*? __100 ng_____

8.5 How much of the toxin is stored*? _50 ug

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

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

**9.0 Insects**

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____

_____

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

_____

_____

**10.0 Plants**

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

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

10.3 What is the origin of the plant? _____

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

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

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

_____

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

_____

_____

_____

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

_____

_____

**11.0 Import Requirements**

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

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

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

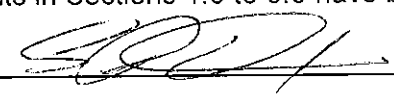
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

## 12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE  _____

## 13.0 Containment Levels

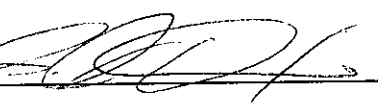
13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 x 2 O 2+ O 3

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

x YES, permit # if on-campus_BIO-UWO-0096_____
O NO, please certify
O NOT REQUIRED for Level 1 containment

## 14.0 Procedures to be Followed

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

SIGNATURE  _____ Date: 11 Feb. 2011

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

__Our lab has a Biohazard level 2 designation and all members are trained to use universal Precautions. We still encouraged all to seek help when starting new projects that use cell lines, toxins or chemicals that they are not entirely familiar with. _____

_____

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

_In the unlikely event of exposure to one of the agents listed above the priority will be the affected person. First aid and medical attention will be given immediately followed by the completion of an incident report. The incident will also be examined in the presence of other lab members to investigate how it occurred and how it can be prevented in the future. _____

**15.0 Approvals**

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

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

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

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

Special Conditions of Approval:

# MSDS FOR ATCC MICROBIAL CULTURES (Biosafety Level 1)

**ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as living microorganisms they are potential biohazards.**

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

**Description**

ATCC microbial cultures consist of all bacteria, fungi, plant and animal viruses, and molecular biology materials such as hosts, vectors, clones and libraries.

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

**SECTION I**
**Hazardous Ingredients**

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

**SECTION II**
**Physical data**

Liquid or solid suspensions; frozen liquid suspensions; freeze-dried.

**SECTION III**
**Health hazards**

This culture is not known to cause disease in healthy human adults or animals.

**SECTION IV**
**Fire and explosion**

Not applicable

**SECTION V**
**Reactivity data**

Stable. Hazardous polymerization will not occur.

## SECTION VI
**Method of disposal**

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

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Dispose of sealed vials of freeze-dried material by dry heat sterilization at 170°C for four hours.

Follow all Federal, State and local regulations.

## SECTION VII
**Special protection information**

**For Biosafety Level 1 Microbial Cultures**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

## SECTION VIII
**Special precautions or comments**

ATCC recommends that all ATCC microbial cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., ASM Press, Washington, DC, 1995), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DNA Molecules** at http://grants.nih.gov/grants/policy/recombinentdnaguidelines.htm.

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

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February 2002

# MSDS FOR GENOMIC DNA

**ATCC genomic DNA products are not hazardous as defined by OSHA 1910.1200.**

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

## SECTION 1. Product Identification

**Product name:** Genomic DNA.

## SECTION 2: Composition/Information on Ingredients

**CAS #:** None

## SECTION 3: Hazards Identification

These products are not known to be hazardous.

## SECTION 4: First Aid Measures

Not applicable

## SECTION 5: Fire Fighting Measures

Stable. Hazardous polymerization will not occur.

## SECTION 6: Accidental Release Measures

Contain the spill and dispose of the material appropriately.

## SECTION 7: Handling and Storage

Store intact at -70°C.

## SECTION 8: Exposure Controls/Personal Protection

Special protection not required under normal usage. Use product in accordance with good laboratory practices.

## SECTION 9: Physical and Chemical Properties

Frozen suspension.

## SECTION 10: Stability and Reactivity

This product is stable.

## SECTION 11: Toxicological Information

Not available.

## SECTION 12: Ecological Information

Not available.

**SECTION 13: Disposal Considerations**
Not available.

**SECTION 14: Transport Information**
Not regulated.

**SECTION 15: Regulatory Information**
Not regulated in the United States.

**SECTION 16: Other Information**

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

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March 2002

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

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

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

**Description**

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

**SECTION I**
**Hazardous Ingredients**

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

**SECTION II**
**Physical data**

Pink or red aqueous liquid

**SECTION III**
**Health hazards**

**For Biosafety Level 1 Cell Lines**

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

**For Biosafety Level 2 Cell Lines**

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

**SECTION IV**
**Fire and explosion**

Not applicable

## SECTION V
**Reactivity data**

Stable. Hazardous polymerization will not occur.

## SECTION VI
**Method of disposal**

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

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

## SECTION VII
**Special protection information**

**For Biosafety Level 1 Cell Lines**

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

**For Biosafety Level 2 Cell Lines**

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

## SECTION VIII
**Special precautions or comments**

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

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

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February 2002

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Information Telephone: (703) 365-2704
Chemtrec (800) 424-9300

Info on Cell Line(s)

Cell Biology

| | | | |
|---|---|---|---|
| ATCC® Number: | **CRL-1573™** | Order this Item | Price: **$279.00** |

| | |
|---|---|
| Designations: | 293 [HEK-293] |
| Depositors: | FL Graham |
| Biosafety Level: | 2 [CELLS CONTAIN ADENOVIRUS ] |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | *Homo sapiens* (human) |
| Morphology: | epithelial |
| Source: | **Organ:** embryonic kidney<br>**Cell Type:** transformed with adenovirus 5 DNA |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| Restrictions: | These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties. |
| Applications: | efficacy testing [92587]<br>transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)<br>viruscide testing [92579] |
| Receptors: | vitronectin, expressed |
| Tumorigenic: | YES |
| DNA Profile (STR): | Amelogenin: X<br>CSF1PO: 11,12<br>D13S317: 12,14<br>D16S539: 9,13<br>D5S818: 8,9<br>D7S820: 11,12<br>THO1: 7,9.3<br>TPOX: 11<br>vWA: 16,19 |
| Cytogenetic Analysis: | |

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Cell Biology

| | | | | |
|---|---|---|---|---|
| ATCC® Number: | **TIB-71™** | Order this Item | Price: | **$279.00** |

| | |
|---|---|
| Designations: | RAW 264.7 |
| Depositors: | WC Raschke |
| Biosafety Level: | 2 |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | *Mus musculus* (mouse) |
| Morphology: | monocyte/macrophage |
| Source: | **Tissue:** ascites<br>**Strain:** BALB/c<br>**Disease:** Abelson murine leukemia virus-induced tumor<br>**Cell Type:** macrophage; Abelson murine leukemia virus transformed |
| Cellular Products: | lysozyme [1207] |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| Applications: | Biological response [92560]<br>transfection host (Roche FuGENE® Transfection Reagents) |
| Receptors: | complement (C3) [1207] |
| Antigen Expression: | H-2d |
| Age: | adult |
| Gender: | male |
| Comments: | This line was established from a tumor induced by Abelson murine leukemia virus. They are negative for surface immunoglobulin (sIg-), Ia (Ia-) and Thy-1.2 (Thy-1.2)This line does not secrete detectable virus particles and is negative in the XC plaque formation assay. The cells will pinocytose neutral red and will phagocytose latex beads and zymosan. They are capable of antibody dependent lysis of sheep erythrocytes and tumor cell targets. LPS or PPD treatment for 2 days stimulates lysis of erythrocytes but not tumor cell targets.Data communicated in Feb. 2007 by Dr Janet W. Hartley, indicates the expression of infectious ecotropic MuLV closely related, if not identical, to the Moloney MuLV helper virus used in the original virus inoculum. The cells also express polytropic MuLV, unsurprisingly based on the mouse passage history of the virus stocks [ PubMed 18177500]. |

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| | | | | |
|---|---|---|---|---|
| ATCC® Number: | **CRL-2593™** | Order this Item | Price: | **$329.00** |
| Designations: | MC3T3-E1 Subclone 4 | | | |
| Depositors: | RT Franceschi | | | |
| Biosafety Level: | 1 | | | |
| Shipped: | frozen | | | |
| Medium & Serum: | See Propagation | | | |
| Growth Properties: | adherent | | | |
| Organism: | *Mus musculus* (mouse) | | | |
| Morphology: | fibroblast | | | |
| Source: | **Organ:** bone<br>**Strain:** C57BL/6<br>**Tissue:** calvaria<br>**Cell Type:** preosteoblast; | | | |
| Cellular Products: | collagen [51540] | | | |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. | | | |
| Tumorigenic: | Yes | | | |
| Age: | newborn newborn | | | |

Comments:

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| | | | |
|---|---|---|---|
| ATCC® Number: | **CCL-61™** Order this Item | Price: | **$279.00** |

| | |
|---|---|
| Designations: | CHO-K1 |
| Depositors: | TT Puck |
| Biosafety Level: | 1 |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | Cricetulus griseus (hamster, Chinese) |
| Morphology: | epithelial-like |
| Source: | **Organ:** ovary |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| Isolation: | **Isolation date:** 1957 |
| Applications: | transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) |
| Virus Resistance: | poliovirus 2; modoc virus; Button Willow virus |
| Cytogenetic Analysis: | Chromosome Frequency Distribution 50 Cells: 2n = 22. Stemline number is hypodiploid. |
| Gender: | female |
| Comments: | The CHO-K1 cell line was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T. T. Puck in 1957. [22224]<br>The cells require proline in the medium for growth. [25976] |
| Propagation: | **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.<br>**Temperature:** 37.0°C |

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| | | | | |
|---|---|---|---|---|
| ATCC® Number: | **CRL-1661™** | Order this Item | Price: | **$329.00** |

| | |
|---|---|
| Designations: | UMR-106 |
| Depositors: | AE Bogden |
| Biosafety Level: | 1 |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | Rattus norvegicus (rat) |
| Morphology: | epithelial |
| Source: | **Organ:** bone<br>**Strain:** Sprague-Dawley<br>**Disease:** osteosarcoma |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| Receptors: | parathyroid hormone (PTH); 1-25(OH)2D3 (bone resorbing steroid hormone) |
| Comments: | The UMR-106 cell line is a clonal derivative of a transplantable rat osteosarcoma that had been induced by injection of radiophosphorous (32P).<br>The cells are responsive to PTH, prostaglandins and bone resorbing steroids.<br>The PTH responsiveness of UMR-106 is greater than that of the related cell line UMR-108 (ATCC CRL-1663).<br>Activation of protein kinase C inhibits ATP induced increases in intracellular calcium levels.<br>Both the original sarcoma and the cloned line were developed by T.J. Martin at the University of Sheffield. |
| Propagation: | **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.<br>**Temperature:** 37.0°C<br>**Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended<br>**Medium Renewal:** 2 to 3 times per week |
| Subculturing: | Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach.<br>Add fresh culture medium, aspirate and dispense into new culture flasks. |

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| | | | | |
|---|---|---|---|---|
| ATCC® Number: | **CRL-1650™** | Order this Item | Price: | **$279.00** |

| | |
|---|---|
| Designations: | COS-1 |
| Depositors: | Y Gluzman |
| Biosafety Level: | 2 [Cells Contain PAPOVAVIRUS ] |
| Shipped: | frozen |
| Medium & Serum: | See Propagation |
| Growth Properties: | adherent |
| Organism: | *Cercopithecus aethiops* |
| Morphology: | fibroblast |
| Source: | **Organ:** kidney<br>**Cell Type:** SV40 transformed |
| Cellular Products: | T antigen |
| Permits/Forms: | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| Applications: | transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) |
| Comments: | This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen. The cells contain a single integrated copy of the complete early region of the SV40 genome. |
| Propagation: | **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.<br>**Atmosphere:** air, 95%; carbon dioxide (CO2), 5%<br>**Temperature:** 37.0°C |

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Comments for pcDNA3:
5446 nucleotides

CMV promoter: bases 209-863
T7 promoter: bases 864-882
Polylinker: bases 889-994
Sp6 promoter: bases 999-1016
BGH poly A: bases 1018-1249
SV40 promoter: bases 1790-2115
SV40 origin of replication: bases 1984-2069
Neomycin ORF: bases 2151-2945
SV40 poly A: bases 3000-3372
ColE1 origin: bases 3632-4305
Ampicillin ORF: bases 4450-5310

Invitrogen
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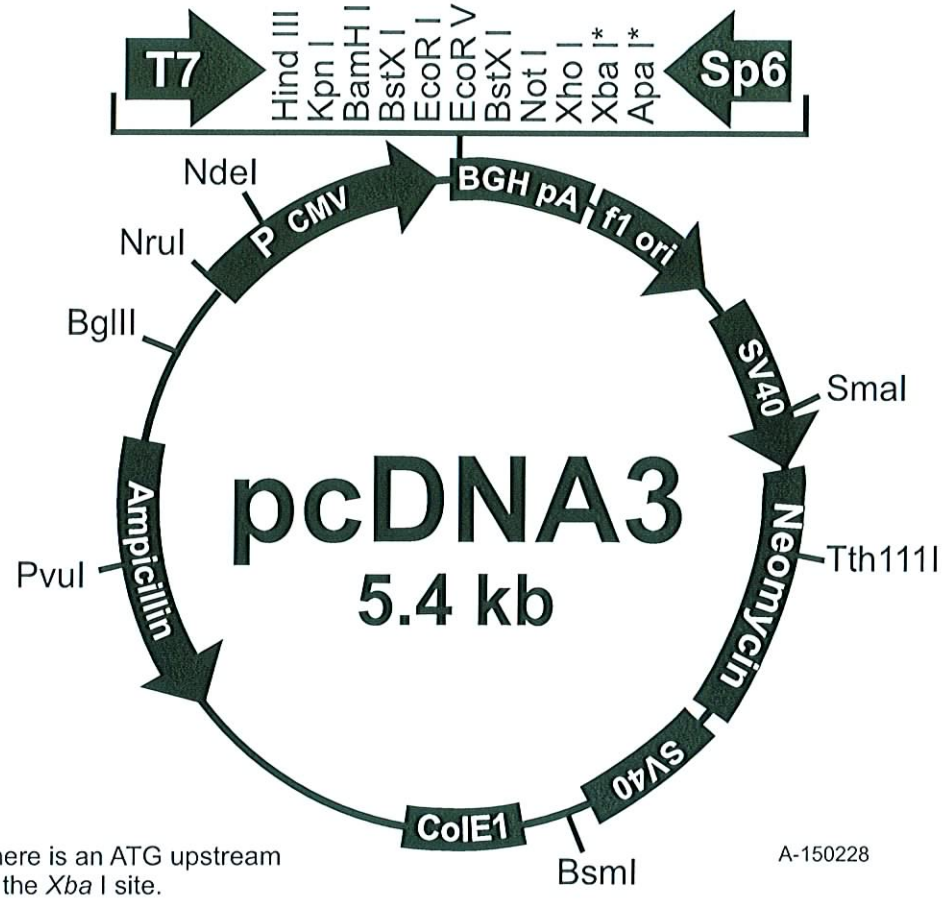
Plasmid (s)




* There is an ATG upstream of the *Xba* I site.

A-150228

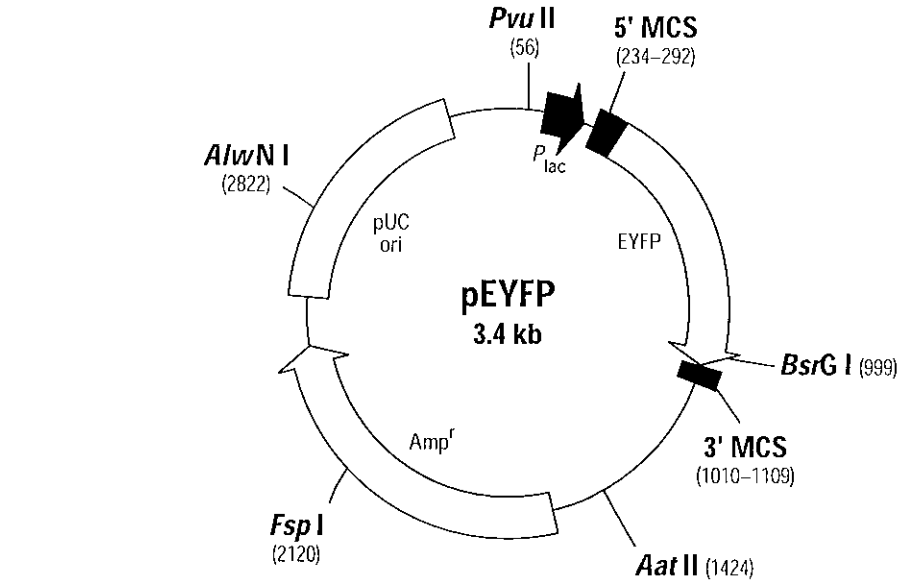
The sequence of pcDNA3 has been compiled from information in sequence databases, published sequences, and other sources. This vector has not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department.

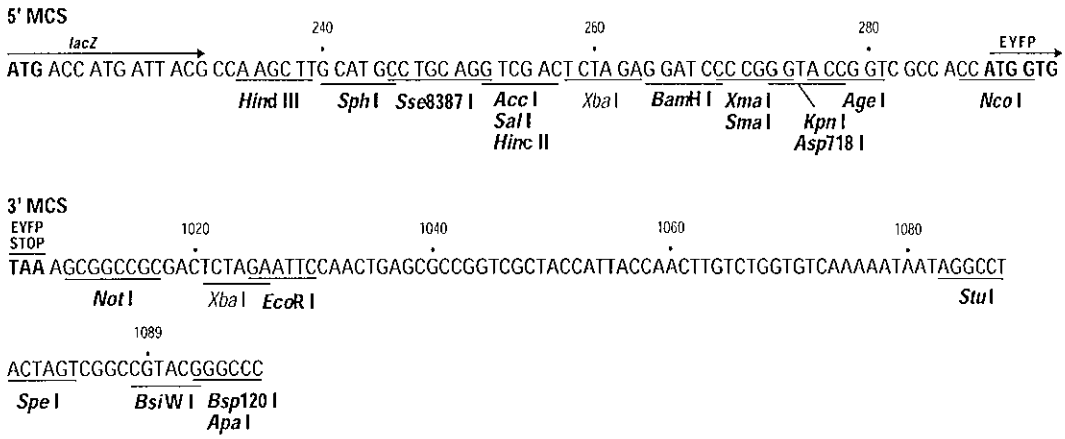
Vector Information

## pEYFP Vector Information

PT3175-5

Catalog #6004-1




**Restriction map and multiple cloning site (MCS) of pEYFP.** Unique restriction sites are in bold. The *Xba* I sites in the 5' and 3' MCSs can be used together to excise the EYFP gene.

### Description:

pEYFP encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm, and the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513 nm, the $E_m$ of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed from EYFP is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

(PR29943; published 03 October 2002)

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The EYFP gene is flanked at the 5' and 3' ends by the two MCSs of the pUC19 derivative pPD16.43 (4). Thus, the EYFP coding sequence can be easily excised from the vector or amplified by PCR. In *E. coli*, EYFP is expressed from the *lac* promoter as a fusion with several additional amino acids, including the first five amino acids of the LacZ protein. Note, however, that if you excise the EYFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., nonfusion) EYFP protein. The pUC19 backbone of EYFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

**Location of features:**

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*–EYFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon: 1006–1008
- 5' multiple cloning site: 234–281
- Enhanced yellow fluorescent protein (EYFP) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 1006–1008
  - Insertion of Val at position 2: 292–294
  - GFP-10C mutations (Ser-65 to Gly: 484–486; Val-68 to Leu: 493–495; Ser-72 to Ala: 505–507; Thr-203 to Tyr: 898–900)
  - His-231 to Leu mutation (A→T): 983
- 3' multiple cloning site: 1010–1109
- Ampicillin resistance gene
  - Promoter: –35 region: 1485–1490; –10 region: 1508–1513
  - Transcription start point: 1520
  - Ribosome binding site: 1543–1547
  - β-lactamase coding sequences
    - Start codon (ATG): 1555–1557; stop codon: 2413–2415
    - β-lactamase signal peptide: 1555–1623
    - β-lactamase mature protein: 1624–2412
- pUC plasmid replication origin: 2563–3206

**Primer location:**

- EGFP-N Sequencing Primer (#6479-1): 355–334
- EGFP-C Sequencing Primer (#6478-1): 942–963

**Propagation in *E. coli*:**

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

**References:**

1. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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

**Notice to Purchaser**

Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

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Vector Information

# Specific EGFP Monoclonal Antibody for Westerns, IP and IC

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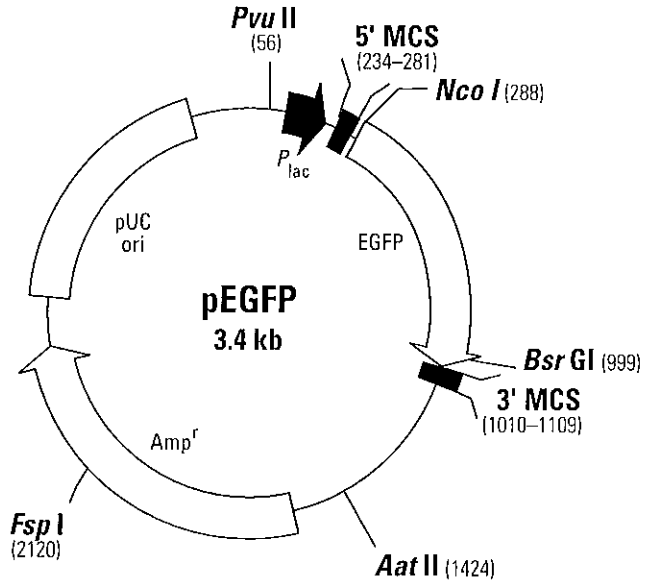
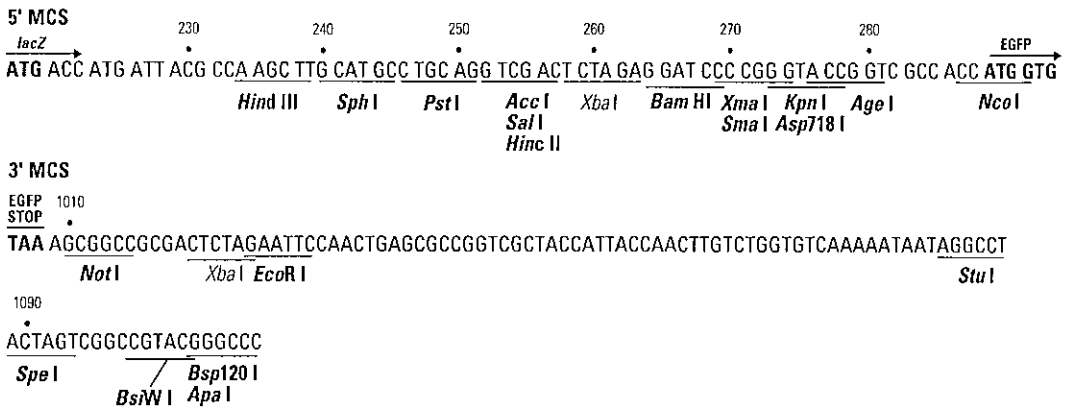
## pEGFP Vector Information

PT3078-5

Catalog #6077-1




**Restriction Map and Multiple Cloning Site (MCS) of pEGFP Vector.** Unique restriction sites are in bold. The *Xba* I sites in the MCS can be used together to excise the EGFP gene.

### Description:

pEGFP carries a red-shifted variant of wild-type green fluorescent protein (GFP) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP encodes the GFPmut1 variant (1) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (2). Upstream sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells.

The EGFP gene was cloned between the two MCS of the pUC19 derivative pPD16.43 (4). The EGFP coding sequence is flanked by separate MCS at the 5' and 3' ends, so the EGFP gene can be easily excised from pEGFP. Alternatively, the EGFP coding sequence can be amplified by PCR. The EGFP gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that a EGFP fusion protein is expressed from the *lac* promoter in *E. coli*. Note, however, that if you excise the EGFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) EGFP protein. The pUC backbone of EGFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.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www.clontech.com



(PR29965; published 03 October 2002)

**Location of features:**

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*–EGFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon: 1006–1008
- 5' Multiple Cloning Site: 234–281
- Enhanced green fluorescent protein (EGFP) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 1006–1008
  - Insertion of Val at position 2: 292–294
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 481–486
  - His-231 to Leu mutation (A→T): 983
- 3' Multiple Cloning Site: 1010–1109
- Ampicillin resistance gene
  - Promoter: –35 region: 1485–1490; –10 region: 1508–1513
  - Transcription start point: 1520
  - Ribosome binding site: 1543–1547
  - β-lactamase coding sequences:
    - Start codon (ATG): 1555–1557; Stop codon: 2413–2415
    - β-lactamase signal peptide: 1555–1623
    - β-lactamase mature protein: 1624–2412
- pUC plasmid replication origin: 2563–3206

**Primer location:**

- EGFP-N Sequencing Primer (#6479-1): 355–334
- EGFP-C Sequencing Primer (#6478-1): 942–963

**Propagation in *E. coli*:**

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

**References:**

1. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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

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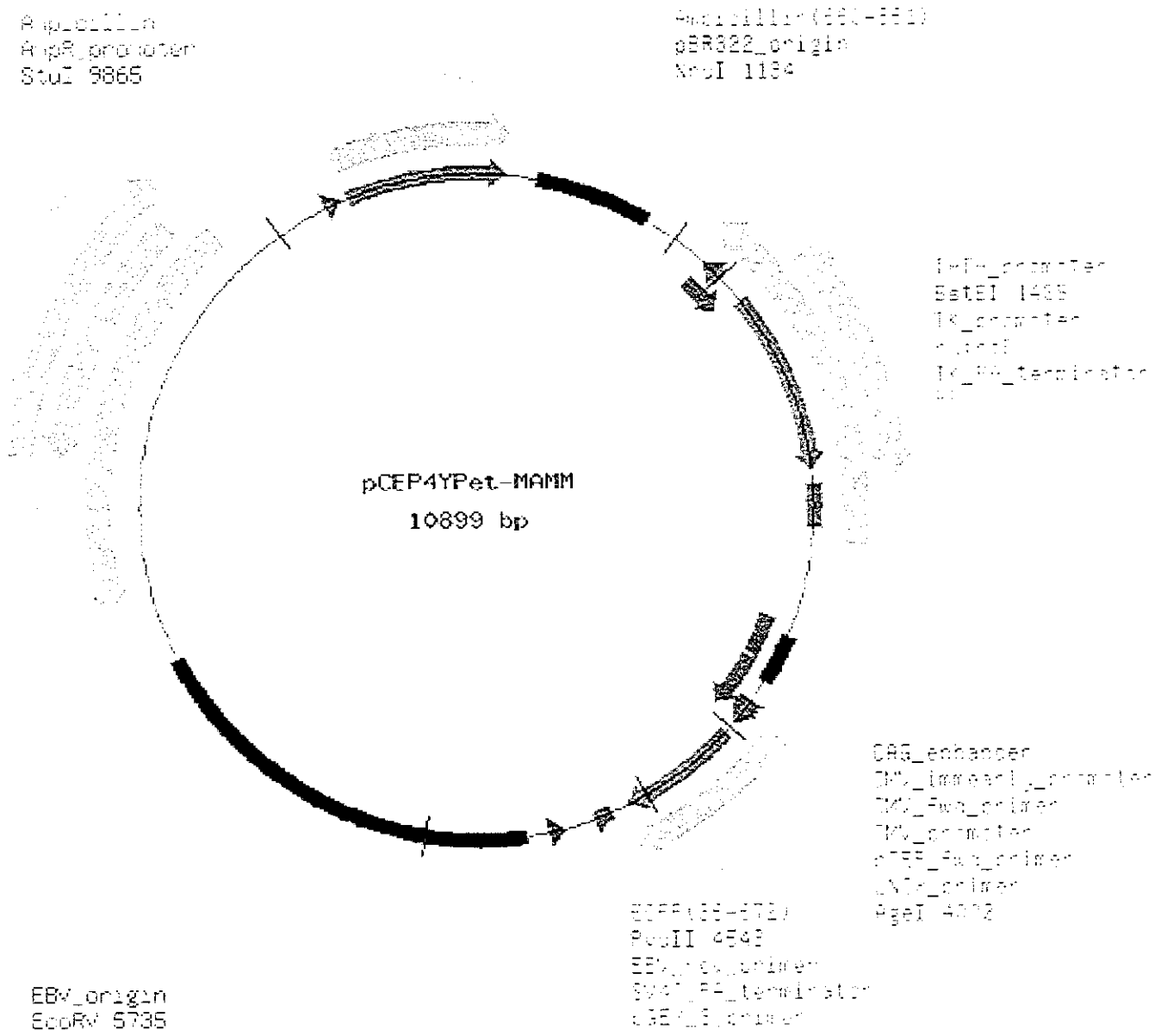
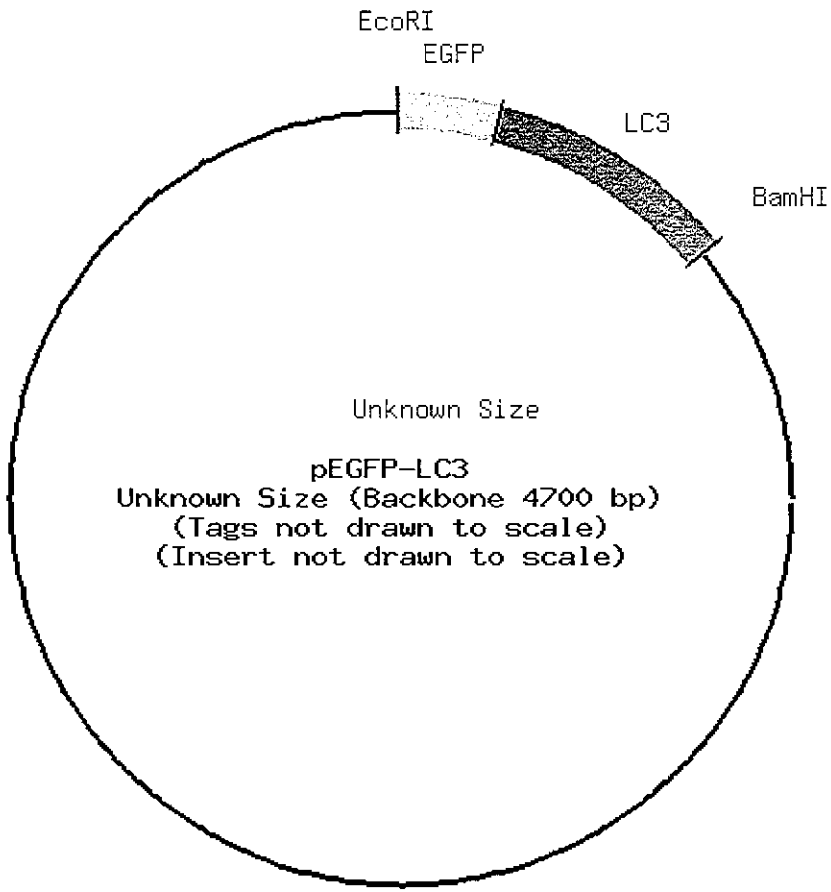

pCEP4YPet-MAMM
10899 bp


EcoRI
EGFP
LC3
BamHI
Unknown Size
pEGFP-LC3
Unknown Size (Backbone 4700 bp)
(Tags not drawn to scale)
(Insert not drawn to scale)

invitrogen™

# Material Safety Data Sheet

**Revision Date:** 21-Jan-2006

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

**Product code** V49320
**Product name** pAd/CMV/V5-DEST™ Gateway® Vector

Vector(s)

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

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

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

## 2. COMPOSITION/INFORMATION ON INGREDIENTS

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

## 3. HAZARDS IDENTIFICATION

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

**Form**
suspension

**Principle Routes of Exposure/ Potential Health effects**

| | |
|---|---|
| **Eyes** | No information available |
| **Skin** | No information available |
| **Inhalation** | No information available |
| **Ingestion** | No information available |

**Specific effects**

| | |
|---|---|
| **Carcinogenic effects** | No information available |
| **Mutagenic effects** | No information available |
| **Reproductive toxicity** | No information available |

| | |
|---|---|
| **Sensitization** | No information available |
| Target Organ Effects | No information available |

## 4. FIRST AID MEASURES

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

## 5. FIRE-FIGHTING MEASURES

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

## 6. ACCIDENTAL RELEASE MEASURES

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

## 7. HANDLING AND STORAGE

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

## 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

| | |
|---|---|
| **Engineering measures** | Ensure adequate ventilation, especially in confined areas |

Personal protective equipment

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

## 9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

| | |
|---|---|
| **Form** | suspension |

Important Health Safety and Environmental Information

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

| | |
|---|---|
| **Water solubility** | No data available |

## 10. STABILITY AND REACTIVITY

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

## 11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

| | |
|---|---|
| **Eyes** | No information available |
| **Skin** | No information available |
| **Inhalation** | No information available |
| **Ingestion** | No information available |

Specific effects

| | |
|---|---|
| **Carcinogenic effects** | No information available |
| **Mutagenic effects** | No information available |
| **Reproductive toxicity** | No information available |
| **Sensitization** | No information available |

Target Organ Effects — No information available

## 12. ECOLOGICAL INFORMATION

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

## 13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

## 14. TRANSPORT INFORMATION

IATA

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

## 15. REGULATORY INFORMATION

**International Inventories**

**U.S. Federal Regulations**

**SARA 313**
Not regulated

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

**U.S. State Regulations**

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

**WHMIS hazard class:**
Non-controlled

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

## 16. OTHER INFORMATION

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

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

End of Safety Data Sheet

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WEBSITE www.listlabs.com

Toxin Info

Product #179A, 179B

# MATERIAL SAFETY DATA SHEET
## Pertussis Toxin
## In Glycerol

Ingredients:

Each vial contains 50.0 µg or 200.0 µg of pertussis toxin (islet-activating protein) at a concentration of 0.2 mg/ml in 50% glycerol, 0.05 M Tris, 0.01 M glycine, 0.5 M sodium chloride, pH 7.5.

Health Hazard Data:

The $LD_{50}$ of pertussis toxin in mice is 18 µg/kg i.p. There is no $LD_{50}$ information for humans.

The excipients have a low hazard for normal industrial uses.

Emergency Procedures:

Pertussis toxin is degraded by the low pH in the gut and is not absorbed. If swallowing occurs, induce vomiting.

If skin pricking should occur, induce bleeding and flush with copious amounts of water. If i.v. or i.m. injection should occur, consult a physician. Attempt to obtain hyperimmune globulin to pertussis from the CDC. In an adult immunized versus whooping cough, no long term ill effects are likely to result.

Handling:

Pertussis toxin, in spite of its name, is not considered hazardous. However, as with any biochemical, it should be handled by trained personnel using good laboratory technique. Observe the following practices when working with pertussis toxin: Special care should be taken when working in conjunction with hypodermic needles. Wear protective gloves, avoid contact with cuts or wounds, avoid inhalation, do not mouth pipet, and flush thoroughly any area of the body that comes in contact with this product. Only individuals who were immunized in childhood against whooping cough should work with this product. This product is intended for research purposes only.

Stability:

Stable for months when stored at -20°C.

Deactivation:

Boil at 100°C for 15 to 30 minutes.

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Western

# TOXIN USE RISK ASSESSMENT

| | |
|---|---|
| **Name of Toxin:** | Pertussis toxin |
| **Proposed Use Dose:** | 0.1 µg |
| **Proposed Storage Dose:** | 50 µg |
| **$LD_{50}$ (species):** | 18000 µg |

**Calculation:**

18000 µg/kg x 50 kg/person

Dose per person based on $LD_{50}$ in µg = 900000

**$LD_{50}$ per person with safety factor of 10 based on $LD_{50}$ in µg =** 90000

**Comments/Recommendations:**

**OK**