

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 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	<u>Stephen M. Sims</u>
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Location of experimental work to be carried out: Building(s) Dental Sciences Room(s) 0074

*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): Role of cytosolic calcium in the regulation of osteoclasts and bone resorption

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Tom Chrones</u>	<u>tchrones@uwo.ca</u>	<u>2006/07/14</u>
<u>Juan Pablo Reyes Valverde</u>	<u>jreyesva@schulich.uwo.ca</u>	<u>2007/08/25</u>
<u>Ryan Shugg</u>	<u>rshugg@uwo.ca</u>	<u>2008/09/01</u>
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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 are all components of the molecular biology reagents needed for expression of various markers in cells	
Vectors/ Plasmids	pcDNA3			
	pEGFP			
	PEYFP			
	pAD/CMV/V5-DEST			
	SV Large T antigen			
	COS			
	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			
	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 solutions 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.
	Tetrodotoxin	Freezer		

Please include a one page research summary or teaching protocol.

Background: Osteoclasts are multinucleated cells responsible for the resorption of bone and calcified cartilage during normal skeletal development and remodeling. Osteoclast-mediated destruction of bone contributes to the pain, progressive deformity and disability associated with rheumatoid arthritis. Osteoclasts also mediate bone loss in osteoporosis and tumor-induced osteolysis. In vivo, the osteoclast alternates between motile and resorbing phenotypes. The resorbing cell is polarized and H⁺ are transported across the ruffled border. The H⁺ pump is electrogenic, thus requiring the movement of counter ions through channels. We have previously identified K⁺ and Cl⁻ channels in osteoclasts, both of which can compensate for H⁺ transport across the ruffled border. Using genetic approaches, Jentsch and co-workers have recently shown that the CIC-7 chloride channel is essential for resorptive activity.

A key factor regulating osteoclast formation, activity and survival is the cytokine RANK ligand (RANKL). RANKL regulates physiological bone remodeling, as well as the destruction of joint tissues in rheumatoid arthritis. Our recent studies have provided novel insights into the early responses of osteoclasts to RANKL. We showed that RANKL causes transient elevation in the concentration of cytosolic free Ca²⁺ ([Ca²⁺]_i), accompanied by activation of outward K⁺ current. The elevation of [Ca²⁺]_i induced by RANKL accelerated activation of NFκB and enhanced osteoclast survival. We have also discovered that RANKL triggers osteoclast chemotaxis. This novel function of RANKL may serve to recruit osteoclasts to sites of physiological and pathological resorption; however, underlying signaling pathways have not been elucidated. Our overall objective is to study the roles of membrane currents and [Ca²⁺]_i in the early responses of osteoclasts to RANKL.

We propose to test the following 3 specific hypotheses.

- 1) Osteoclast Cl⁻ current is regulated by RANKL and other factors.
- 2) RANKL activates K⁺ currents in osteoclasts, contributing to resorptive activity and survival.
- 3) Subcellular Ca²⁺ changes are essential for RANKL-induced osteoclast chemotaxis.

Summary of research plan: We will isolate osteoclasts from the long bones of neonatal rats and rabbits using established methods. Ion currents will be recorded using patch clamp and [Ca²⁺]_i using fluorescence ratio imaging techniques. Biophysical properties of Cl⁻ and K⁺ currents will be evaluated using voltage-clamp protocols with which we have extensive experience. Channels will be identified based on voltage-dependence, reversal potential, ion substitution, sensitivity to selective blockers and, in some cases, RT-PCR of microisolated osteoclasts. Resorptive activity will be determined using an in vitro pit formation assay. We anticipate that certain channels reside in endomembranes and traffic to the cell surface during formation of the ruffled border. Accordingly, we will stimulate osteoclasts with extracellular matrix components and RANKL to induce formation of the ruffled border and quantify changes in Cl⁻ and K⁺ currents. Channel distribution will be assessed by confocal microscopy of osteoclasts labeled by immunofluorescence or following transfection with GFP-tagged channels. Cl⁻ channels will be activated using hypotonic medium and ligands including RANKL. The involvement of CIC-7 in mediating Cl⁻ currents will be assessed using osteoclast-like cells derived from osteopetrotic patients bearing CIC-7 mutations. Preliminary studies reveal at least 2 types of K⁺ channels activated by RANKL. We will identify these channels and investigate the mechanisms underlying their activation. The roles of these channels in mediating the effects of RANKL on osteoclast survival and resorptive activity will be addressed using specific blockers and control compounds. RANKL-induced osteoclast chemotaxis will be characterized, including the role of subcellular changes of [Ca²⁺]_i and K⁺ channel activity. Changes in [Ca²⁺]_i will be suppressed using the intracellular chelator BAPTA. The downstream effectors of Ca²⁺ will be identified.

Significance: Information regarding the regulation of osteoclast channels and motility by RANKL is important for understanding the pathways regulating osteoclast formation, activation and survival. The proposed studies may lead to the development of novel therapies directed at arresting the loss of bone in metabolic, inflammatory and neoplastic diseases.

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:

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)	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	1.5		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Recombinant Adenovirus - Non replicating	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.15	Invitrogen	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

Level 2 ↑

*Please attach a Material Safety Data Sheet or equivalent

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	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Rats, Mice	2008-043-06
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	HEK 293	Invitrogen, ATCC
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	RAW 264.7, CHO	ATCC
Non-human primate	<input checked="" type="radio"/> Yes <input type="radio"/> No	COS-1	ATCC
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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 1 2 2+ 3

3.0 Use of Human Source Materials

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

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

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

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
<i>Escherichia Coli</i> (TOP 10 competent cells-Invitrogen)	pcDNA3 pEGFP PEYFP pAD/CMV/V5-DEST SV Large T antigen COS pEGFP-LC3 pCEP4YPet-MAMM YPET			

* 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? 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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

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

6.5 Will the agent(s) be shed by the animal: YES 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)? YES No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

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

8.3 What is the LD₅₀ (specify species) of the toxin__ (a) 5-30mg/kg and (b) 3ng/kg _____

8.4 How much of the toxin is handled at one time*? __ (a) 10 ug and (b) 100 ng _____

8.5 How much of the toxin is stored*? __ (a) 1 mg and (b) 50 ug (this is the maximum available to purchase)_

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

*For information on biosecurity requirements, please see:
<http://www.uwo.ca/humanresources/docandform/docs/health>

Pertussis Vaccination

9.0 Insects

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____
 "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? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? YES 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? YES 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? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

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

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

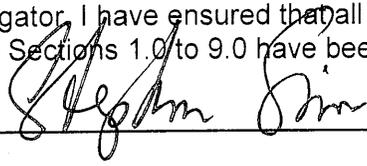
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus_BIO-UWO-0096 _____
 NO, please certify
 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 Alghm Sm Date: Sept 30th 2010

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

One Shot® TOP10 Electrocomp™ *E. coli*

Cat. Nos. C4040-50 (10 reactions); C4040-52 (20 reactions)

Store at -80°C

Caution

This product contains irritants and may be harmful if swallowed. Review the Material Safety Data Sheet before handling.

Description

TOP10 *E. coli* are provided at a transformation efficiency of 1×10^{10} cfu/µg supercoiled DNA and are ideal for high-efficiency cloning and plasmid propagation. They allow stable replication of high-copy number plasmids. The genotype of TOP10 Cells is similar to the DH10B™ strain.

Components Supplied

Component	Amount
TOP10 Electrocomp™ <i>E. coli</i> Cells	21 x 50 µl
pUC19 Control DNA (10 pg/µl)	50 µl
S.O.C. Medium	6 ml

Genotype

F *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*Δ*M15* Δ*lacX74* *recA1 araD139* Δ(*ara-leu*) 7697 *galU galK rpsL* (Str^R) *endA1 nupG* λ-

General Guidelines

Follow these guidelines when using One Shot® TOP10 Electrocomp™ *E. coli*.

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Thaw One Shot® competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently.
- One Shot® TOP10 cells do not require IPTG to induce expression from the *lac* promoter. If blue/white screening is required to select for transformants, make sure that selective plates contain 50 µg/ml X-gal.

Part No. C40405.pps

Rev. Date: 8 Aug 2006

For technical support, email tech_support@invitrogen.com.
For country-specific contact information, visit www.invitrogen.com.

Transforming Competent Cells

Perform the following before starting the transformation procedure:

- Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
- Spread X-gal onto LB agar plates containing antibiotic, if desired.
- Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.
- Place cuvettes on ice and set up your electroporator for bacterial transformation as per the manufacturer's instructions.
- One 15 ml snap-cap tube per transformation

Transformation Procedure

Use this procedure to transform One Shot® TOP10 Electrocomp™ *E. coli*. We recommend including the pUC19 control plasmid DNA supplied with the kit (10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation experiment to verify the efficiency of the competent cells. **Do not** use these cells for chemically competent transformation.

1. Thaw, on ice, one vial of One Shot® TOP10 Electrocomp™ cells for each transformation.
2. Add 1-2 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and mix gently. For the pUC19 control, add 10 pg (1 µl) of DNA into a separate vial of One Shot® cells and mix gently.
3. Transfer the cells to the chilled electroporation cuvette on ice.
4. Electroporate the cells as per the manufacturer's recommended protocol.
5. Aseptically add 250 µl of pre-warmed S.O.C. Medium to each vial.
6. Transfer the solution to a 15 ml snap-cap tube and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance gene.

- Spread 10 to 150 μl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:50 into LB Medium (e.g. remove 20 μl of the transformation mix and add to 980 μl of LB Medium) and plate 20-100 μl .
- Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
- Invert the selective plate(s) and incubate at 37°C overnight.
- Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Calculating Transformation Efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per μg of plasmid DNA. Remember that the total volume of the transformation mixture is 300 μl .

Transformation efficiency (# transformants/ μg DNA) =

$$\frac{\# \text{ of colonies}}{10 \text{ pg pUC19}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{300 \mu\text{l total volume}}{X \mu\text{l plated}} \times \text{dilution factor}$$

For example, if transformation of 10 pg of pUC19 DNA yields 100 colonies when 30 μl of a 1:50 dilution is plated, then the transformation efficiency is:

$$\frac{100 \text{ colonies}}{10 \text{ pg DNA}} \times \frac{10^6 \text{ pg}}{\mu\text{g}} \times \frac{300 \mu\text{l total vol.}}{30 \mu\text{l plated}} \times 50 = 5 \times 10^9$$

Quality Control

Each lot of TOP10 competent cells is tested for transformation efficiency using the control plasmid included in the kit and the protocol on page 2. Test transformations are performed on 3 to 20 vials per lot, depending on batch size. Transformed cultures are plated on LB plates containing 100 $\mu\text{g}/\text{ml}$ ampicillin and incubated overnight. Transformation efficiency should be $\sim 1 \times 10^{10}$ cfu/ μg plasmid DNA. In addition, untransformed cells are tested for the appropriate antibiotic sensitivity and the absence of phage contamination.

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For research use only. Not intended for any animal or human therapeutic or diagnostic use.



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

Product code 500257
Product name TOP 10 - ONE SHOT

Company/Undertaking Identification

INVITROGEN CORPORATION
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 Suspension

**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

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

May be harmful if swallowed.

Specific effects

Carcinogenic effects
Mutagenic effects
Reproductive toxicity
Sensitization

(Long Term Effects)

No information available
No information available
No information available
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

Hazard Class

Subsidiary Class

Packing group

UN-No

Not classified as dangerous in the meaning of transport regulations
No information available
No information available
No information available
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 contain 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

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

Cell Line(s)

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

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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MSDS FOR ATCC MICROBIAL CULTURES (Biosafety Level 2)

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 may cause disease in humans 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 2 Microbial Cultures**

Handle as biohazardous material under Biosafety Level 2 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>.

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MATERIAL SAFETY DATA SHEET

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

Cell Biology

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

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

Morphology:



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

Permits/Forms:

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

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

Receptors: vitronectin, expressed

Tumorigenic: YES

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

Cytogenetic Analysis:

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Cell Biology

ATCC® Number: **TIB-71™** [Order this Item](#) Price: **\$269.00**

Designations: RAW 264.7

Depositors: WC Raschke

Biosafety Level: 2

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Mus musculus* (mouse)
monocyte/macrophage

Morphology:



Tissue: ascites

Strain: BALB/c

Source: **Disease:** Abelson murine leukemia virus-induced tumor
Cell Type: macrophage; Abelson murine leukemia virus transformed

Cellular Products: lysozyme [[1207](#)]

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](#)]
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].

Related Links ▶

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Cell Biology

ATCC® Number: **CRL-1650™** Price: **\$275.00**

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
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%.
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%
Temperature: 37.0°C

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Plasmid 14032: pCEP4YPet-MAMM

Gene/insert name: YPet
Insert size (bp): Unknown
Species of gene(s): Other
Relevant mutations/deletions: Mammalian optimized YPet
Vector backbone: pCEP4
([Search Vector Database](#))
Type of vector: Mammalian expression
Backbone size (bp): 10410
Cloning site 5': Unknown
Site destroyed during cloning: Unknown
Cloning site 3': Unknown
Site destroyed during cloning: Unknown
5' Sequencing primer: See map ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Sequence: Visit www.addgene.org/14032
Plasmid Provided In: DH5a
Principal Investigator: Patrick Daugherty

Comments: The assembled sequence for this plasmid is inaccurate. The plasmid is actually 2.3kb smaller than the sequence suggests.

Article: [Evolutionary optimization of fluorescent proteins for intracellular FRET](#). Nguyen AW et al. (Nat Biotechnol. 2005 Mar . 23(3):355-60. [Pubmed](#))

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

Also, please include the text "Addgene plasmid 14032" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/14032 for updated plasmid information and related links.

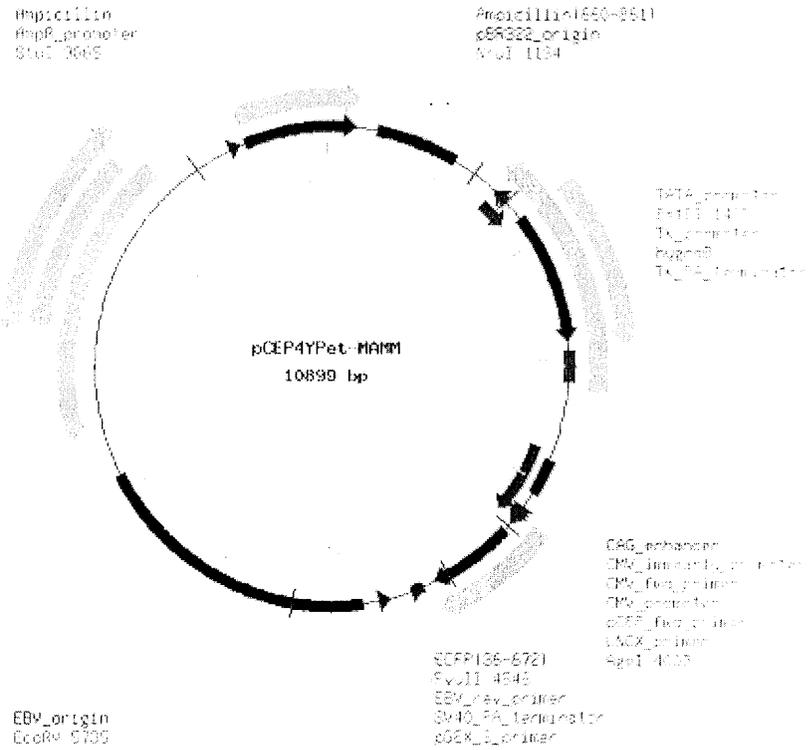
Page 1 of 2 - Date: 09/27/2010

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Plasmid(s) Info



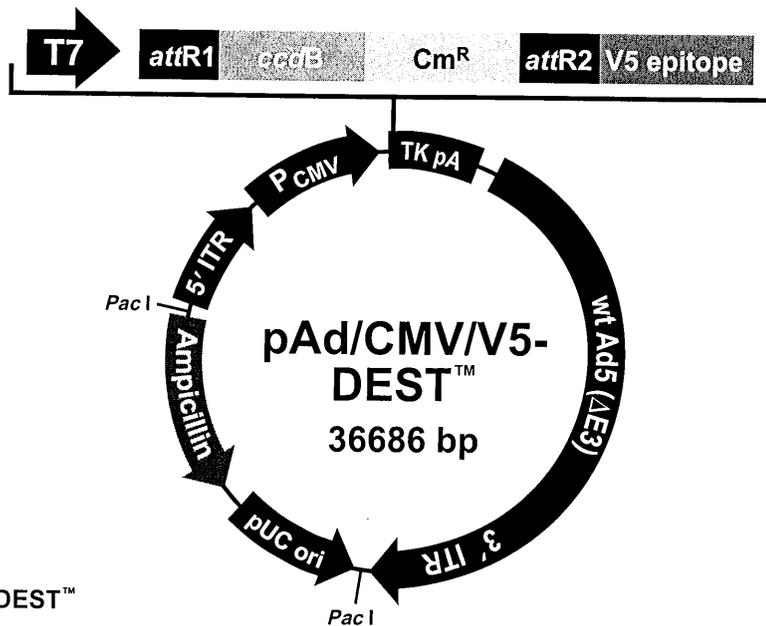
Find this plasmid at: www.addgene.org
Enter "14032" in the search box



Please check www.addgene.org/14032 for updated plasmid information and related links.

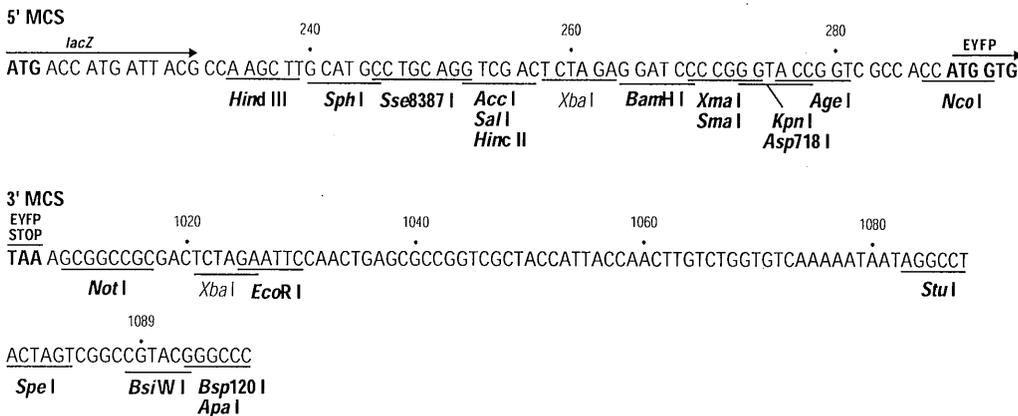
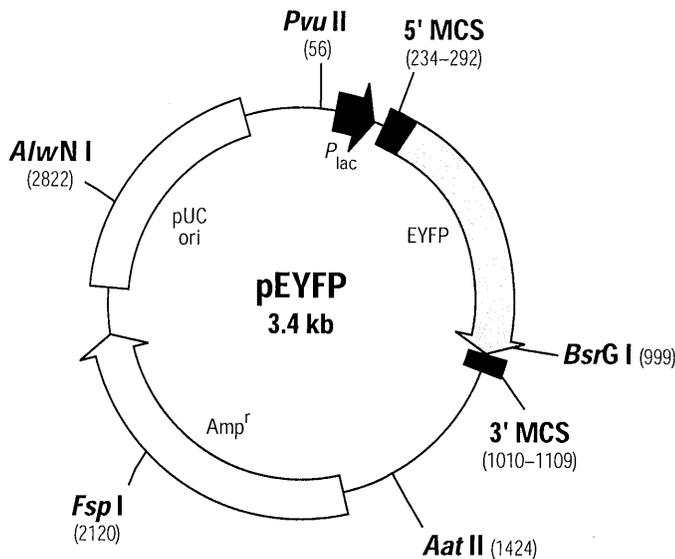
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**Comments for pAd/CMV/V5-DEST™
36686 nucleotides**

- Human Ad5 sequences (wt 1-458; includes 5' L-ITR and packaging signal): 1-458
 - pAd forward priming site: bases 361-384
 - CMV promoter: bases 728-1315
 - T7 promoter/priming site: bases 1359-1378
 - attR1 site: bases 1407-1531
 - ccdB gene: bases 1960-2265 (C)
 - Chloramphenicol resistance gene (Cm^R): bases 2607-3266 (C)
 - attR2 site: bases 3547-3671
 - V5 epitope: bases 3697-3738
 - TK polyadenylation signal: bases 3765-4036
 - Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3' R-ITR): bases 4056-34604
 - pAd reverse priming site: bases 4059-4082
 - pUC origin: bases 34781-35442 (C)
 - Ampicillin (*bla*) resistance gene: bases 35568-36428 (C)
 - bla* promoter: bases 36429-36527 (C)
 - Pac I restrictions sites: bases 34610 and 36684
- (C) = complementary strand



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 μ g/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.



Find this plasmid at: www.addgene.org
Enter "11546" in the search box

Plasmid 11546: EGFP-LC3

Gene/insert name: EGFP-LC3B
 Alternative names: NM_022818
 Human homolog of yeast ATG8p
 Insert size (bp): 380
 GenBank/Entrez ID of insert: NM_022818
 Gene/insert aliases: MAP1LC3B, LC3B, MAP1A/1BLC3
 Species of gene(s): H. sapiens (human)
 Fusion proteins or tags: EGFP
 Terminal: N terminal on insert
 Vector backbone: pEGFP-C3
 ([Search Vector Database](#))
 Backbone manufacturer: Clontech
 Type of vector: Mammalian expression
 Backbone size (bp): 4727
 Cloning site 5': EcoRI
 Site destroyed during cloning: No
 Cloning site 3': EcoRI
 Site destroyed during cloning: No
 5' Sequencing primer: CMV Forward ([List of Sequencing Primers](#))
 3' Sequencing primer: EBV Reverse
 Bacteria resistance: Kanamycin
 High or low copy: Unknown
 Grow in standard E. coli @ 37C: Yes
 Selectable markers: Neomycin
 If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid: Kopito Lab - see reference
 Sequence: Visit www.addgene.org/11546
 Plasmid Provided In: DH5a
 Principal Investigator: Karla Kirkegaard

Article: [Subversion of cellular autophagosomal machinery by RNA viruses](#). Jackson WT et al. (PLoS Biol. 2005 May . 3(5):e156. [Pubmed](#))

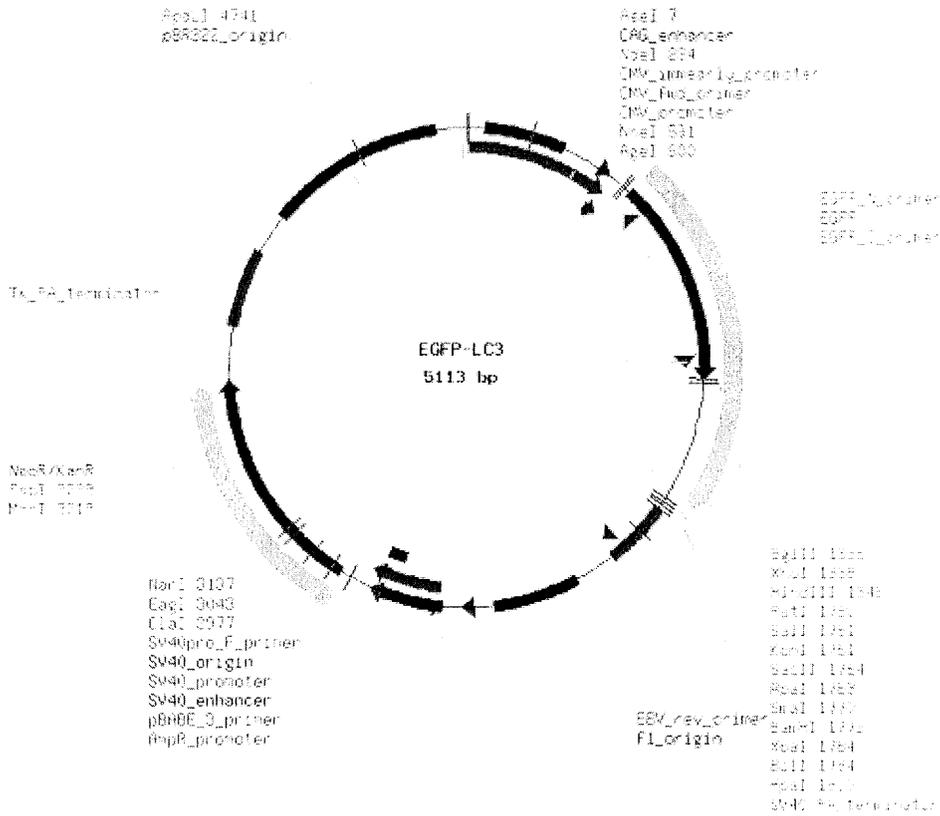
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11546" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11546 for updated plasmid information and related links.



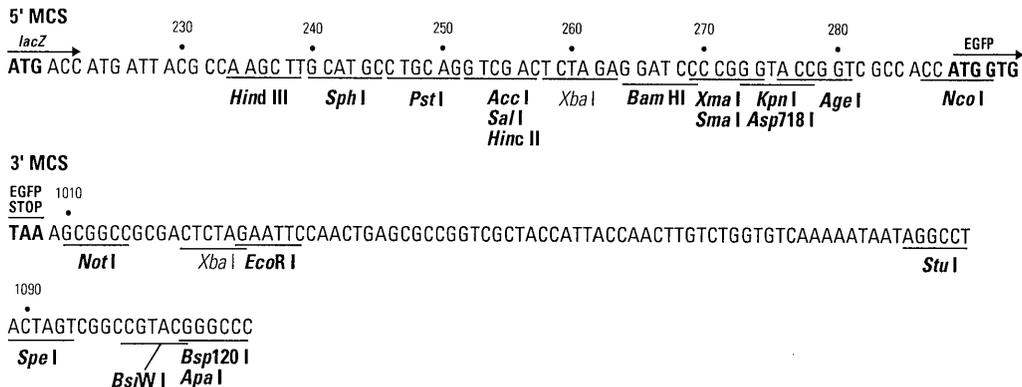
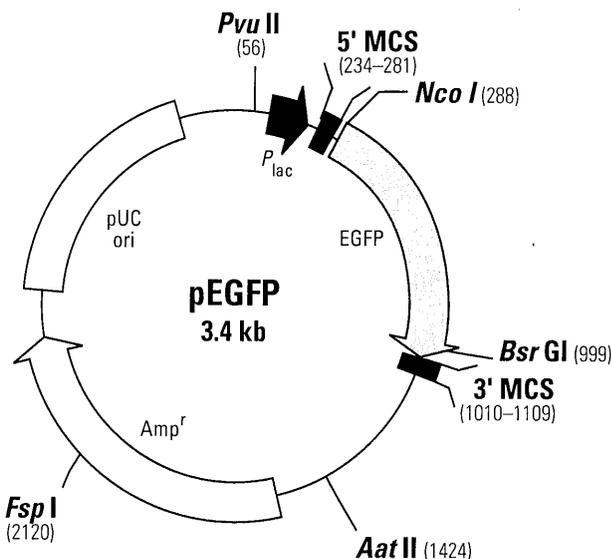
Find this plasmid at: www.addgene.org
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Restriction Map and Multiple Cloning Site (MCS) of pEGFP Vector. Unique restriction sites are in bold. The *Xba* I sites in the MCS can be used together to excise the EGFP gene.

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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(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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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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Plasmid 13031: pcDNA3-EGFP

Gene/insert name: Enhanced Green Fluorescent Protein
Alternative names: EGFP
GFP
Insert size (bp): 700
Vector backbone: pcDNA3
([Search Vector Database](#))
Type of vector: Mammalian expression
Backbone size (bp): 5446
Cloning site 5': XhoI
Site destroyed during cloning: No
Cloning site 3': XbaI
Site destroyed during cloning: No
5' Sequencing primer: T7 ([List of Sequencing Primers](#))
3' Sequencing primer: GTCTTGTAGTTGCCGTCGTC
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Selectable markers: Neomycin
If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid: EGFP from discontinued clonetech vector.
Sequence: Visit www.addgene.org/13031
Plasmid Provided In: DH5a
Principal Investigator: Doug Golenbock

Comments: The primer for sequencing out the 5' end of the GFP based constructs (GFP/EGFP, CFP, YFP) is 5'- GTCTTGTAGTTGCCGTCGTC -3'

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

Also, please include the text "Addgene plasmid 13031" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/13031 for updated plasmid information and related links.

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

Product code 54357
Product name PUC-19 Control DNA

Company/Undertaking Identification

INVITROGEN CORPORATION
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
Eye contact

Wash off immediately with plenty of water. If symptoms persist, call a physician.
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 Extremely soluble in water.

10. STABILITY AND REACTIVITY

Stability

Stable

Materials to avoid

No information available

Hazardous decomposition

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

Mutagenic effects

Reproductive toxicity

Sensitization

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 contain 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



Toxin(s) Info

TOXIN USE RISK ASSESSMENT

TOXIN: Tetrodotoxin

PROPOSED USE (DOSE): 10 ug (use), 1 mg (storage)

LD₅₀ (species): 5 mg/kg

CALCULATION:

5000 ug/kg X 70 kg/person = 350,000 ug per person

Divide by safety factor(s) of 10 (as applicable): 35,000 ug per person

COMMENTS/RECOMMENDATION:

LD₅₀

35000 ug/
person

>>

10 ug (use)

1000 ug (storage)

o, OK

