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	Stephen M. Sims
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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:

Name	UWO E-mail Address	Date of Biosafety Training
Tom Chrones	tchrones@uwo.ca	2006/07/14
Juan Pablo Reyes Valverde	jreyesva@schulich.uwo.ca	2007/08/25
Ryan Shugg	rshugg@uwo.ca	2008/09/01
Ben Wheal	bwheal@uwo.ca	2009/09/16
Natsuko Tanabe	ntanabe@uwo.ca	2008/02/18
Alexey Pereverzev	alexey.pereverzev@schulich.uwo.ca	2006/07/13

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			
	NFAT/AP-1 3x luciferase			
	pGL3-NFAT luciferase			
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 ClC-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 ClC-7 in mediating Cl- currents will be assessed using osteoclast-like cells derived from osteopetrotic patients bearing ClC-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? 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 O No	x Yes O No	x Yes O No	1.5		x 1 O 2 O 2+ O 3
Recombinant Adenovirus - Non replicating	O Yes x No	O Yes x No	O Yes x No	0.15	Invitrogen	O 1 x 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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	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	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 O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 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	addgene	eGFP	These plasmids are all used to express and produce large amounts of the protein of interest in the transfected cells
	pEGFP	addgene	eGFP	
	PEYFP	addgene	eYFP (GFP variant)	
	NFAT/AP-1 3x luciferase	addgene	NFAT	
	pGL3-NFAT luciferase	addgene	NFAT	
	pEGFP-LC3	addgene	MAP1LC3B, LC3B	
	pCEP4YPet-MAMM YPET	addgene	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? 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 actin-EGFP and EGFP proteins that can be visualized. Cells are 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)_(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 LD_{50} (specify species) of the toxin__(a) 5-30mg/kg and (b) 18ug/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? 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 _____

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 Stephan Sin Date: May 6 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:

invitrogen

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

Genotype

F *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*) 7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G 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.

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.

7. 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.
8. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
9. Invert the selective plate(s) and incubate at 37°C overnight.
10. 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) =

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

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:

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

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 µg/ml ampicillin and incubated overnight. Transformation efficiency should be ~1 x 10^{10} cfu/µg plasmid DNA. In addition, untransformed cells are tested for the appropriate antibiotic sensitivity and the absence of phage contamination.

invitrogen Material Safety Data Sheet Revision Date: 01-Mar-2010

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

Product code 500257
Product name TOP 10 - ONE SHOT

Company/Undertaking Identification

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

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

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

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

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

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

3. HAZARDS IDENTIFICATION

Form
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

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	May be harmful if swallowed.

Specific effects (Long Term Effects)

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

Target Organ Effects: No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

For research use only

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

End of Safety Data Sheet

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.

February 2002

ATCC®

MATERIAL SAFETY DATA SHEET

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.

February 2002

ATCC

MATERIAL SAFETY DATA SHEET

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.

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.

February 2002

ATCC

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.

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)
Morphology:	epithelial PHOTO
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	

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BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

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)
Morphology:	monocyte/macrophage 
Source:	**Tissue:** ascites **Strain:** BALB/c **Disease:** Abelson murine leukemia virus-induced tumor **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] 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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Related Cell Culture Products

Login Required
Product Information Sheet

Cell Biology

ATCC® Number:	CRL-1650™ Order this Item	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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BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

Bio-materials management; basic repository to complex partnership-level services

BioStandards

Biological Reference Material and Consensus Standards for the life science community

addgene

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

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.

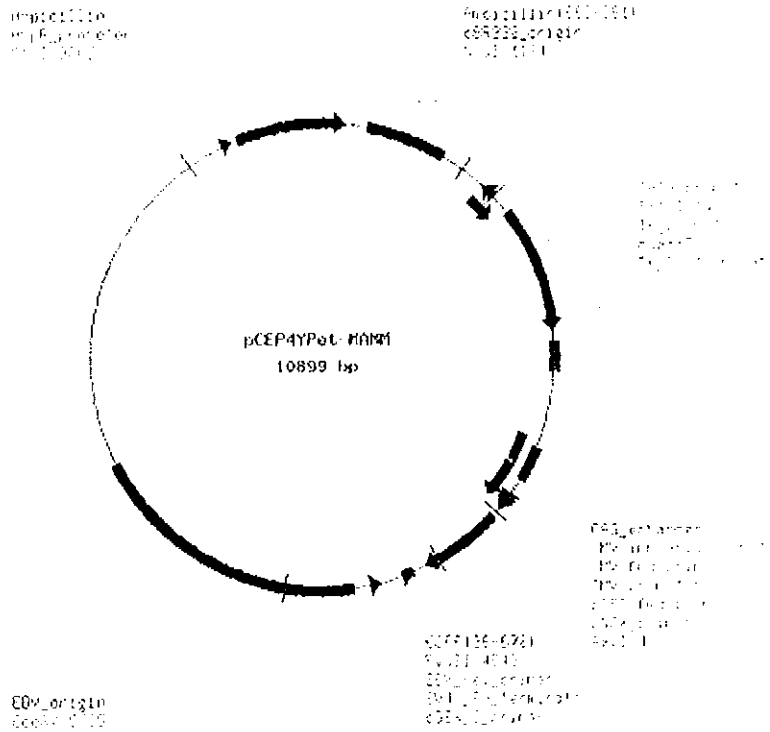
Also, please include the text "Addgene plasmid 14032" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

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

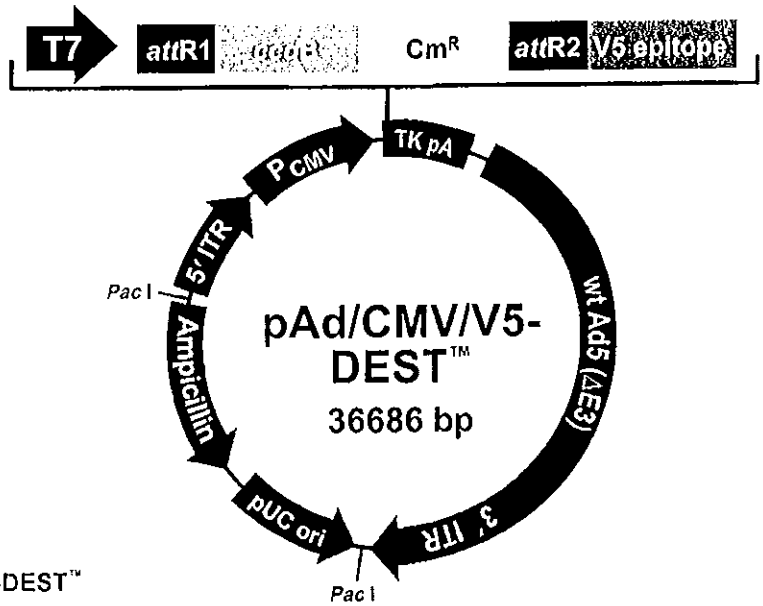
Plamid(s) Info

addgene

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.



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
*att*R1 site: bases 1407-1531
*ccd*B gene: bases 1960-2265 (C)
Chloramphenicol resistance gene (Cm^R): bases 2607-3266 (C)
*att*R2 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

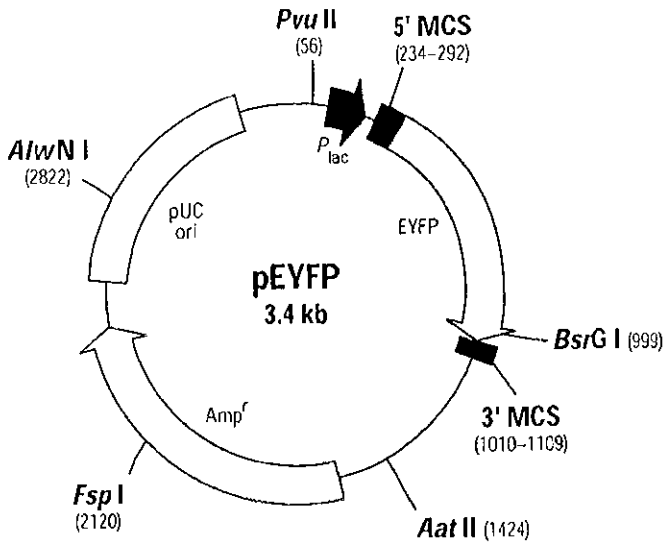
Invitrogen™
life technologies

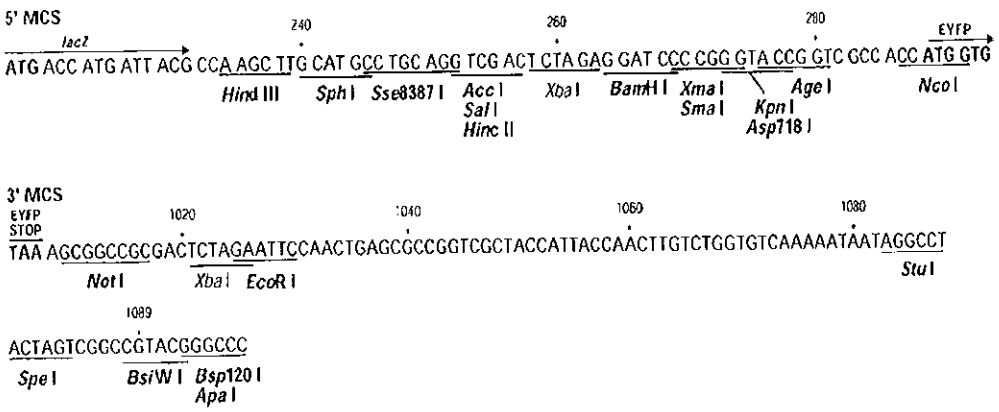
Vector Information

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)

Clontech

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www.clontech.com

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.

addgene

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)

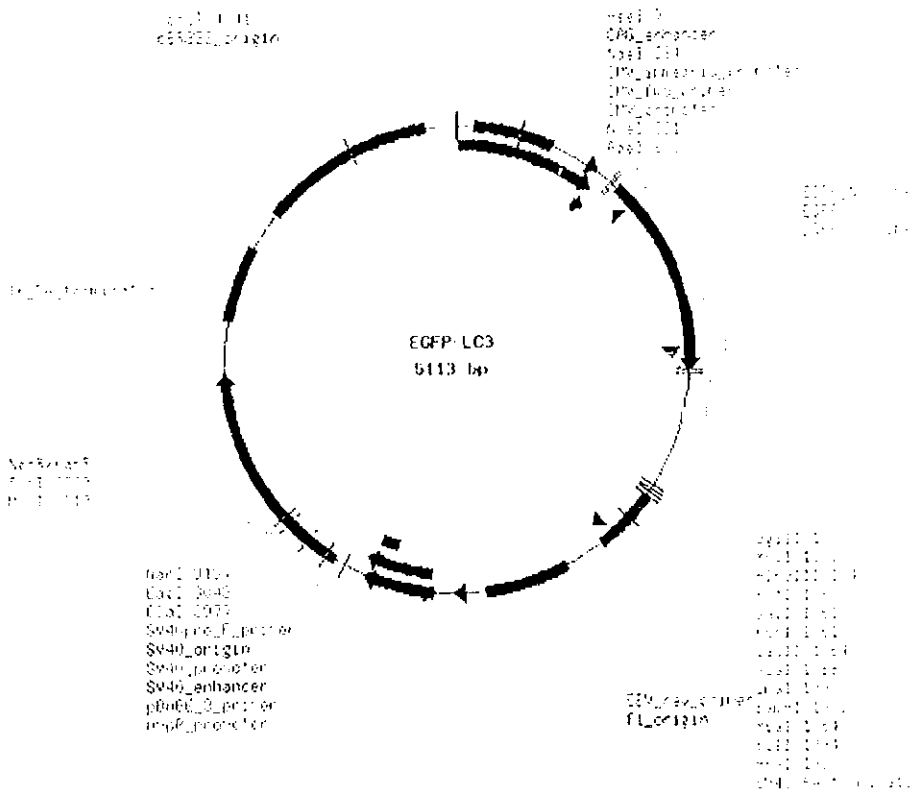
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

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.

addgene

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



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

Vector Information

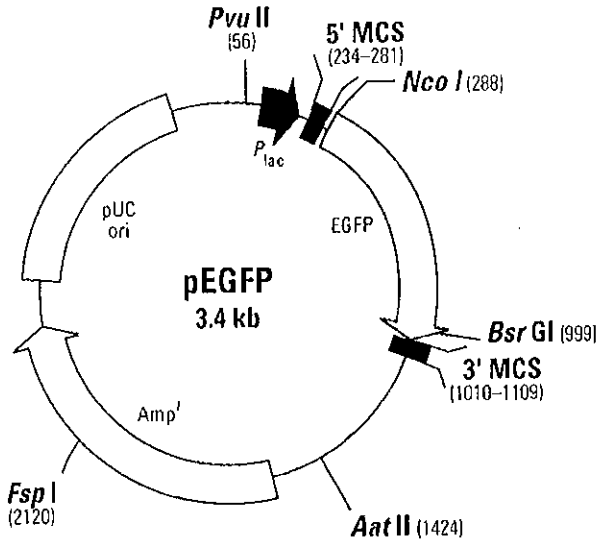
Specific EGFP Monoclonal Antibody for Westerns, IP and IC

Visit our Website for more details! click here...

pEGFP Vector Information

PT3078-5

Catalog #6077-1



5' MCS

lacZ →

ATG ACC ATG ATT ACG CCA AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG GTC GCC ACC ATG GTG

230 240 250 260 270 280

Hind III Sph I Pst I Acc I Sal I Hinc II Xba I BamHI Xma I Sma I Kpn I Asp718 I Age I Nco I

EGFP →

3' MCS

EGFP STOP 1010

TAA AGCGGCCGCGACTCTAGAATTCCAACTGAGCGCCGGTCGCTACCATTACCAACTTGTCTGGTGTCAAAAATAATAGGCCT

Not I Xba I EcoR I Stu I

1090

ACTAGTCGGCCGTACGGGCCC

Spe I BsiW I Bsp120 I Apa I

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*.

Clontech

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Technical Support (US)
E-mail: tech@clontech.com
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.

addgene

Find this plasmid at: www.addgene.org
Enter "13031" in the search box

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'

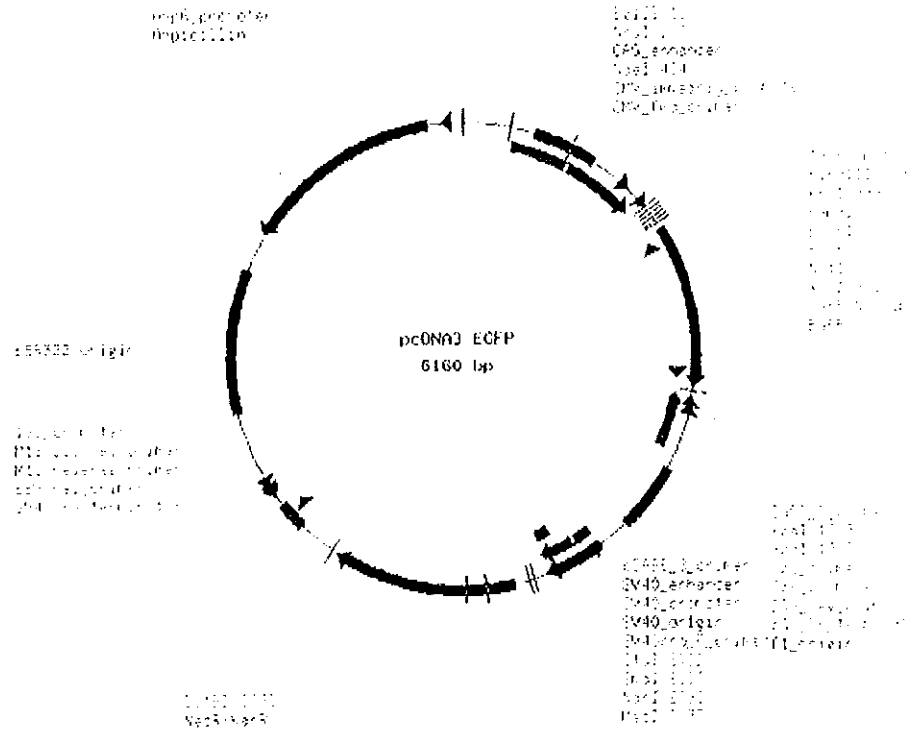
Please acknowledge the principal investigator if you use this plasmid in a publication.

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.

addgene

Find this plasmid at: www.addgene.org
Enter "13031" in the search box



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

invitrogen

Material Safety Data Sheet

Revision Date: 01-Mar-2010

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

Product code 54357
Product name pUC 19 Control DNA

Company/Undertaking Identification

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

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

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

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

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

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

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

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

Specific effects

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

Target Organ Effects: No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits
Engineering measures: Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form: Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	Extremely soluble in water.	

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

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

Specific effects (Long Term Effects)

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

Target Organ Effects: No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

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

U.S. State Regulations

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

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

For research use only

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

End of Safety Data Sheet

Western

Toxin(s) Info

TOXIN USE RISK ASSESSMENT

TOXIN: Tetrodotoxin

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

LD_{50} (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:

LD_{50}

35000 ug/person >> 10 µg (use)

1000 µg (storage)

OK

Western

TOXIN USE RISK ASSESSMENT

Pertussis Vaccination

TOXIN: Pertussis toxin

PROPOSED USE (DOSE): 100 ng or 0.1 µg (use), 50 µg (storage)

LD_{50} (species): 3 ng/kg or 0.003 µg/kg

CALCULATION:

0.003 ug/kg X 70 kg/person = 0.21 ug per person

Divide by safety factor(s) of 10 (as applicable): 0.021 ug per person

COMMENTS/RECOMMENDATION:

LD_{50} (per person)
0.021 ng

LL

0.1 ng (use)
50 ng (storage)

∴ LD_{50} exceeded

addgene

Find this plasmid at: www.addgene.org
Enter "17870" in the search box

Plasmid 17870: pGL3-NFAT luciferase

Gene/insert name:	3x NFAT binding sequence
Alternative names:	NF-AT site
Insert size (bp):	Unknown
Species of gene(s):	Other
Fusion proteins or tags:	IL2 promoter
Terminal:	C terminal on insert
Fusion proteins or tags:	Luciferase
Terminal:	C terminal on backbone
Vector backbone:	pGL3 (Search Vector Database)
Backbone manufacturer:	Promega
Type of vector:	Mammalian expression,Luciferase
Backbone size (bp):	4800
5' Sequencing primer:	n/a (List of Sequencing Primers)
3' Sequencing primer:	LucNrev
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/17870
Author's Map:	Visit www.addgene.org/17870
Plasmid Provided In:	DH5a
Principal Investigator:	Jerry Crabtree

Comments: This plasmid has three copies of the NF-AT site cloned upstream of the minimal IL-2 promoter from -89 to +51.

Article: Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Clipstone NA et al. (Nature. 1992 Jun 25. 357(6380):695-7. Pubmed)

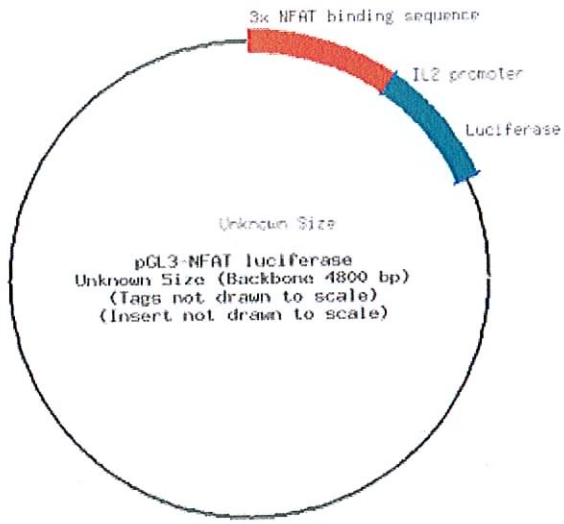
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 17870" 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/17870 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "17870" in the search box



Please check www.addgene.org/17870 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "11783" in the search box

Plasmid 11783: NFAT/AP-1 3x luciferase

Gene/insert name:	NFAT/AP-1 3x
Alternative names:	IL-2 promoter
Insert size (bp):	120
Gene/insert aliases:	Il2, Il-2
Species of gene(s):	M. musculus (mouse)
Fusion proteins or tags:	luciferase
Terminal:	C terminal on backbone
Vector backbone:	pT81Luc (Search Vector Database)
Backbone manufacturer:	ATCC
Type of vector:	Luciferase,Bacterial expression
Backbone size (bp):	Unknown
Cloning site 5':	HindIII
Site destroyed during cloning:	No
Cloning site 3':	XhoI
Site destroyed during cloning:	No
5' Sequencing primer:	LucNrev (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid:	DJ McKean (Hedin, et al., 1997, PMID 9548483)
Sequence:	Visit www.addgene.org/11783
Plasmid Provided In:	DH5a
Principal Investigator:	Anjana Rao

Comments: Three tandem repeats of the NFAT/AP-1 binding site at approximately -287 of the murine IL-2 promotor. Sequence: 5'-AGCTTGGATCC AAGAGGAAAA TTTGTTTCAT ACAGAAGGCG TTAAGAGGAA AATTTGTTTC ATACAGAAGG CGTTAAGAGG AAAATTTGTT TCATACAGAA GGCGTTCAAG CTTGTCGAC-3  .

Article: Gene expression elicited by NFAT in the presence or absence of cooperative recruitment of Fos and Jun. Macian F et al. (EMBO J. 2000 Sep 1. 19(17):4783-95. Pubmed)

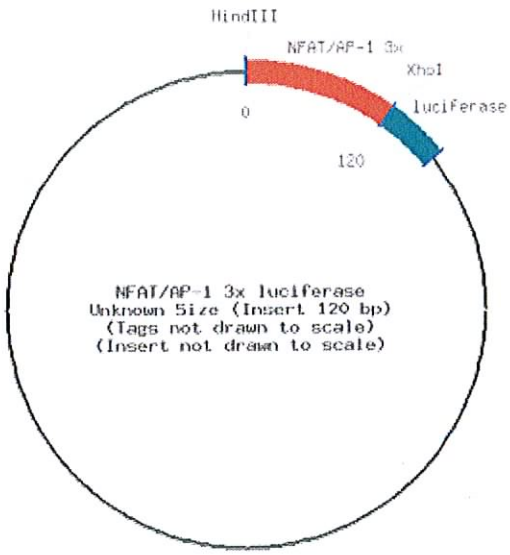
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11783" 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/11783 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "11783" in the search box



Please check www.addgene.org/11783 for updated plasmid information and related links.