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

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

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

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

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

PRINCIPAL INVESTIGATOR	Bryan Heit
DEPARTMENT	Microbiology & Immunology
ADDRESS	Room H320, Health Sciences Addition
PHONE NUMBER	519-661-3407 (internal: x83407)
EMERGENCY PHONE NUMBER(S)	226-374-1574 (cell)
EMAIL	bheit@uwo.ca

Location of experimental work to be carried out: Building(s) Health Sciences Addition Room(s): H316, H310

*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:HSFC (applied), NSERC (applied), UWO startup funding (received)
GRANT TITLE(S): Molecular mechanisms regulating apoptotic body phagocytosis, and its impact on atherosclerosis, inflammation and antigen presentation.

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

Name	UWO E-mail Address	Date of Biosafety Training

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.

Mammalian Cell Lines:

My lab will use several common cell lines which originated from mammalian sources, including humans. In all cases these cell lines are considered BSL2 biohazards. These cells will be cultured using a biosafety hood and HEPA-filtered CO2 incubator, by users following standard BSL2 safety procedures. For long-term storage these cells will be cryofrozen, and kept in a liquid nitrogen doer. All cell culture procedures and liquid nitrogen storage will be in room H310. Some experimental procedures (fixing, staining for microscopy/FACS, western blotting) will take place in room H316. Used/unneeded cells will be destroyed through the addition of a minimum of 5% v/v bleach before disposal.

The following human cell lines will be used:

- •HeLa
- •U937
- •HEK293
- •THP-1
- •Mono Mac 6
- •HL-60
- •Daudi
- •Riji
- •Jurkat T-cell

The following cell lines will be used, with the source species indicated in [square brackets]

- •RAW264.7 [mouse]
- •CHO [hamster]
- •J774 [mouse]
- •BV-2 [mouse]

Bacterial Cells Carrying Plasmids

My laboratory will frequently culture bacteria for the purpose of producing, propagating and storing DNA constructs, and for production of recombinant proteins. The DH5α strain of *Escherichia coli* will be used for production/propagation of DNA constructs, while the BL21 strain of *Escherichia coli* will be used for the production of recombinant proteins. Both of these *Escherichia coli* strains are BLS1 organisms, and will be handled using standard BSL1 handling protocols. Culturing of these organisms will take place in room H316 and in the common bacterial culture facility, located in room (xyz) of the dental sciences building. Purification of DNA constructs and proteins will be performed in room H316. Bacterial cultures will be stored as glycerol stocks, in a -80C freezer located in room H310. Liquid bacterial waste will be sanitized by autoclaving before disposal. Bacteria on agar plates will be disposed of <xyz>.

Tissue Culture Media:

My laboratory will use tissue culture media, some types which contain between 5% and 10% fetal bovine serum (FBS). FBS is a potential biohazard. To minimize the risk associated with FBS, all FBS-containing media will be prepared and aliquoted in a biosafety hood. All FBS-containing liquid waste will either be autoclaved or sanitized using 5% v/v bleach before disposal. All solid waste that has contacted FBS will be autocalved prior to disposal. FBS and FBC containing materials will be handled in rooms H310 and H316.

Recombinant Proteins:

On occasion my laboratory will have recombinant proteins, produced either in-lab, or purchased from commercial sources. Recombinant proteins will be stored in frozen aliquots at either -20C or -80C until needed. Fluids containing these proteins will be autoclaved or sanitized using 5% v/v bleach before disposal. All solid waste that has contacted these proteins will be autocalved prior to disposal. These materials will be handled in rooms H310 and H316.

Please include a one page research summary or teaching protocol.

Apoptosis, the controlled demolition of old, unneeded, infected or damaged cells, is fundamental to homeostasis and immunity. Each day billions of cells in our body undergo apoptosis, wherein the cellular contents of dying cells are degraded and packaged into membrane bound vesicles termed apoptotic bodies. Apoptotic bodies serve a dual purpose: they prevent the spillage of cellular contents into the extracellular milieu, while simultaneously packaging cell contents into particles small enough to be internalized by professional phagocytes. The clearance of apoptotic bodies by phagocytes – termed efferocytosis – is required for tissue homeostasis, with failure to clear these particles leading to inflammation, autoimmunity and neurodegenerative diseases. If not cleared promptly, apoptotic bodies rupture and release their contents in a process termed secondary necrosis. Because these intracellular contents include pro-inflammatory substances such as nucleotides (ATP, UTP), secondary necrosis promotes inflammation. Indeed, the defective removal of apoptotic cells is an initiating event in inflammatory disorders such as atherosclerosis and neurodegenerative diseases such as Alzhimers. While it is unclear if secondary necrosis drives autoimmunity, it is well established that the presentation of antigens derived from apoptotic cells plays a central role in maintaining self-tolerance, with failures in this system leading to autoimmunity. The regulation of apoptosis and subsequent clearance of apoptotic cells also contributes to immunity against infectious agents such as viruses and intracellular bacteria. Efferocytosis of apoptotic bodies released by infected cells allows for the processing and presentation of intracellular pathogen-derived antigens by professional phagocytes. These phagocytes then transport these normally sequestered antigens to lymphatic tissues, where the antigens are the presented on MHC II, thus driving the formation of adaptive immunity. Despite the obvious importance of efferocytosis, little is known about the process itself. Efferocytosis is a three step process, consisting of an initial recognition of the apoptotic body, internalization of the apoptotic body by a phagocyte, and finally, destruction of the apoptotic body. My research program will aim to understand the signalling which regulates these processes, with a focus on the receptors that bind apoptotic bodies and the signalling these receptors induce. This research is being conducted using two human diseases as model of the efferocytic process.

While the initial aims in my proposal are intended to identify the ligands, receptors and signalling underlying efferocytosis, the long-term goals of my research program will be to understand the role of efferocytosis in antigen presentation and atherosclerosis. To this end I have developed two long term projects which build upon my initial three aims. The first of these projects will seek to understand how phagocytes "decide" between presenting efferosome-derived antigens in an immunostimulatory verses tolerogenic fashion. Despite the fact that the same efferocytic process takes up apoptotic bodies derived from uninfected cells and cells containing intracellular pathogens, the antigens contained in those efferosomes must be presented to the adaptive immune system in vastly different manners; with immunogenic antigen presentation being required to produce immunity against an intracellular pathogen, but leading to autoimmunity in the case of non-infected cells. My second long-term goal is to understand why efferocytosis is defective in atherosclerosis. The failure to clear apoptotic cells from the vascular intima is considered to be a major initiating factor in the formation of atherosclerotic lesions. New targets for clinical intervention may be identified through understanding how the atherogenic environment impairs efferocytosis. This later study is of particular interest, as similar efferocytic defects are observed in several other chronic inflammatory disorders, including neurodegenerative disorders such as Alzheimer's, inflammatory disorders such as IBD, and autoimmune disorders such as lupus.

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)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli DH5α	O Yes X No	O Yes X No	O Yes X No	1.0L	Dr. Sergio Grinstein	X 1 O 2 O 2+ O 3
E. coli BL21	O Yes X No	O Yes X No	O Yes X No	1.0L	Dr. Sergio Grinstein	X 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
	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 Ø No N/A		Not applicable
Rodent	O Yes Ø No N/A		
Non-human primate	O Yes Ø No N/A		
Other (specify)	O Yes Ø No N/A		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	X Yes O No	HeLa, U937, HEK293, THP-1, Mono Mac 6, HL-60, Daudi, Riji, Jurkat	All lines: 2	All but Mono Mac 6: ATCC Mono Mac 6: DSMZ BH
Rodent	X Yes O No	RAW264.7, CHO, J774, BV-2	All lines: 2 except 2.	ATCC BH
Non-human primate	O Yes Ø No	BH		
Other (specify)	O Yes Ø No	BH		

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

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required X 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/UNKNOWN**	**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? O 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 tranfection
E.Coli DH5α	Please see appendix	Please see appendix	Please see appendix	Please see appendix

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below X 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 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 O YES X NO
- E1A oncogene X YES ~~O NO~~
- Known oncogenes X YES, please specify See appendix O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

4.5 Will virus be replication defective? O YES X NO

4.6 Will virus be infectious to humans or animals? O YES X NO 9 H

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 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

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

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

_____

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) Cholera Toxin Subunit B
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 Unknown, thought to be non-toxic (see MSDS)

8.4 How much of the toxin is handled at one time*? 1-10µl of a 1mg/ml solution

8.5 How much of the toxin is stored*? 0.5mg, in a 1mg/ml solution at -80°C

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? X YES, please give country of origin: USA O NO
If no, please proceed to Section 12.0

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

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

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

BBA Am not ready to order cells, will require permits before placing orders.

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.

Lynn Hunt

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?
O YES, date of most recent biosafety inspection: _____
X NO, please certify
O NOT REQUIRED for Level 1 containment

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

14.0 Procedures to be Followed

2.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
None.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
Follow standard UWO/BSL2 protocols appropriate for the reagent.

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

SIGNATURE Date: August 8, 2011.

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:

Cell Line Info

ATCC

MATERIAL SAFETY DATA SHEET

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

MATERIAL SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

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

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

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

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

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

SECTION 3 - HAZARD IDENTIFICATION

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

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

Health Hazards

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

See next page for Biosafety Level 2 cell cultures.

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

SECTION 4 - FIRST AID MEASURES

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

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

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

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

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

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available

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

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

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

SECTION 6 - ACCIDENTAL RELEASE MEASURES

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

Methods for Cleaning Up

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

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

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

SECTION 7 - HANDLING AND STORAGE

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

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

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

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

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

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

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

Sensitization
Skin: Data not available
Respiratory: Data not available

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

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

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

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

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

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

Contact ATCC for regulatory information.

SECTION 16 - OTHER INFORMATION

ATCC

MATERIAL SAFETY DATA SHEET

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

Cell Biology

ATCC® Number:	**CCL-2™** Order this Item	Price:	**$279.00**

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
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 ([21491] Nucleofection technology from Lonza Roche Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18

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

Login Required
Product Information Sheet

Cell Biology

ATCC® Number:	**CRL-1593.2™** Order this Item	Price:	**$279.00**

Designations:	U-937
Depositors:	H Koren
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	monocyte
Source:	**Disease:** histiocytic lymphoma
Cellular Products:	lysozyme; beta-2-microglobulin (beta 2 microglobulin); tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-alpha, TNF alpha), after stimulation with phorbol myristic acid (PMA)
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:	The original U-937 cell line was established by Dr. K. Nilsson's laboratory in 1974 and he has requested the following: (1) In all papers reporting any use of this cell line or any derivatives thereof a direct reference should be made to Sundstrom and Nilsson (Int. J. Cancer 17: 565-577, 1976). (2) Any proposed commercial use of the cells should be negotiated with Professor Kenneth Nilsson, Rudbeck Laboratory, SE-751 85 Uppsala, Sweden. (3) No distribution of any of the cells or sublines derived therefrom should be made to third parties; (4) The cells should be used for non-clinical, non-commercial research only.
Isolation:	**Isolation date:** 1974
Applications:	transfection host (Nucleofection technology from Lonza Roche Transfection Reagents)
Receptors:	complement (C3)
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 10,12 D16S539: 12 D5S818: 12 D7S820: 9,11 THO1: 6, 9.3 TPOX: 8,11 vWA: 14, 15

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Cell Biology

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

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

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Cell Biology

ATCC® Number:	**TIB-202™**	Order this Item	Price:	**$279.00**

Designations:	THP-1
Depositors:	S Tsuchiya
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	monocyte 
Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;
Cellular Products:	lysozyme [58053]
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 Transfection Reagents)
Receptors:	complement (C3), expressed [58053] Fc, expressed
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16
Age:	1 year infant
Gender:	male
Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053] Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]

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ATCC® Number:	**CCL-240™**	Order this Item	Price:	**$279.00**

Designations:	HL-60
Depositors:	RC Gallo
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	myeloblastic 
Source:	**Organ:** peripheral blood **Disease:** acute promyelocytic leukemia **Cell Type:** promyeloblast;
Cellular Products:	tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-alpha, TNF alpha), after stimulation with phorbol myristic acid [23403]
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 Transfection Reagents)
Receptors:	complement, expressed [1050] Fc, expressed [1050]
Tumorigenic:	Yes
Oncogene:	myc +
DNA Profile (STR):	Amelogenin: X CSF1PO: 13,14 D13S317: 8,11 D16S539: 11 D5S818: 12 D7S820: 11,12 THO1: 7,8 TPOX: 8,11 vWA: 16
Cytogenetic Analysis:	The stemline chromosome number is pseudodiploid with the 2S component occurring at 6.2%. Five markers (M2 through M6) were common to most S metaphases. DM's, which varied in numbers per cell, occurred in all metaphases karyotyped. HSR chromosomes were not detected.

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Cell Biology

ATCC® Number:	**CCL-213™**	Order this Item	Price:	**$279.00**

Designations:	Daudi
Depositors:	G Klein
Isotype:	IgM
Biosafety Level:	2 [Cells Contain HERPESVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Organ:** peripheral blood **Disease:** Burkitt's lymphoma **Cell Type:** B lymphoblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** May, 1967
Applications:	transfection host (Roche Transfection Reagents)
Receptors:	complement, expressed Fc, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 11,12 D16S539: 10,12 D5S818: 8,13 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 15,17
Cytogenetic Analysis:	Male human karyotype with stemline number of 46. The karyotype is diploid in 66% of the cells and is stable within the stemline.
Isoenzymes:	G6PD, B
Age:	16 years
Gender:	male
Ethnicity:	Black

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ATCC® Number:	**TIB-152™**	Order this Item	Price:	**$279.00**

Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast 
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]
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 Transfection Reagents)
Receptors:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.
Gender:	male

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Cell Biology

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

Designations:	RAW 264.7
Depositors:	WC Raschke
Biosafety Level:	2
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	monocyte/macrophage 
Source:	**Tissue:** ascites **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 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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Cell Biology

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

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

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ATCC® Number:	**TIB-67™**	Order this Item	Price:	**$279.00**

Designations:	J774A.1
Depositors:	P Ralph
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	macrophage 
Source:	**Tissue:** ascites **Strain:** BALB/cN **Disease:** reticulum cell sarcoma **Cell Type:** monocyte/macrophage macrophage;
Cellular Products:	interleukin 1 beta lysozyme [1080]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	La Jolla California, United States **Isolation date:** 1968
Applications:	Biological response [92560] transfection host (Roche Transfection Reagents)
Receptors:	complement (C3), expressed [1135] Fc receptor, IgG, high affinity I (Fcgr1), expressed [13710]
Age:	adult
Gender:	female
Comments:	J774A.1 cells are active in antibody dependent phagocytosis [Pubmed: 1101071]. Their growth is inhibited by dextran sulfate, PPD and LPS [Pubmed: 318922]. They synthesize large amounts of lysozyme and exhibits minor cytolysis but predominantly antibody-dependent phagocytosis. Interleukin 1 beta (Il1b) is synthesized continuously by this line.
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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Section 4

Appendix to Biological Agents Registry Forms: Genetically Modified Cell Lines

My work will make extensive use of cells transiently transfected with plasmids carrying transgenes which either:

a) Demark a subcellular structure
b) Indicate the activity of a signaling pathway
c) Modify cellular behavior or signaling pathway

All of these plasmids are non-integrating plasmids which lack a mammalian replication site. As such they represent a minimal risk to individuals in the lab as they persist in cells for only short periods of time. Regardless, some plasmids have a potential risk in that they carry genes which may be oncogenic, or otherwise alter cellular function. These are listed below, categorized by the theoretical impact they may have on cells.

ONCOGENES AND GENES AFFECTING CELL SURVIVAL OR REPLICATION:
These constructs, when expressed in cells, have the potential to reduce control over cell division, or alter the cells response to survival/apoptosis signals.

Transgene	Label*	Effect on Cell
CLBD	GFP/RFP	Inhibition of apoptosis
PTEN	YFP	Tumor suppressor, overexpression suppresses cell division and cell survival
PDK1	GFP	Increased cell-survival signaling
Cyc1	GFP/mCherry	Increased susceptibility to apoptosis
AKT	None**	Increases cell survival
Rac1	GFP/RFP	Potential oncogene
Rac2	CFP	Potential oncogene
Rac3	CFP	Potential oncogene
CDC42	GFP/RFP/CFP	Potential oncogene
TC10	CFP	Known oncogenic enhancer
Bad	GFP/mCherry	Overexpression enhances sensitivity to apoptosis
Bax	GFP/mCherry	Overexpression enhances sensitivity to apoptosis
Bid/tBid	GFP/mCherry	Overexpression enhances sensitivity to apoptosis
Caspase 8	GFP/mCherry	Overexpression enhances sensitivity to apoptosis
Ran	CFP	Potential oncogene, controls DNA replication
HRas	GFP/RFP	Known oncogene
KRas	GFP/RFP	Known oncogene
NRas	GFP/RFP	Known oncogene
Src	GFP/RFP	Known proto-oncogene
Syk	GFP	Known proto-oncogene
Pak1	GFP/YFP/Myc	Known oncogene

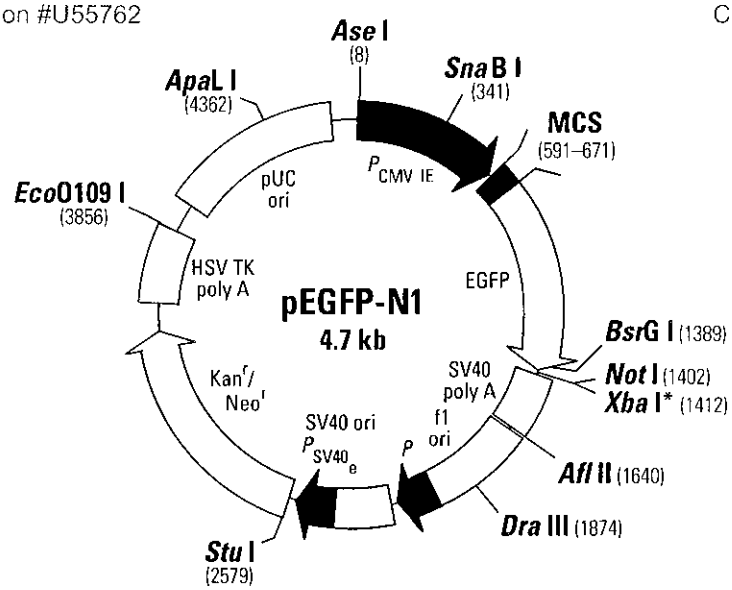
* All plasmids are in the pEGFP vectors, or a variant thereof where the EGFP is replaced with another fluorescent protein.
** in pEGFP-N1, with stop codon imposed between Akt and eGFP.

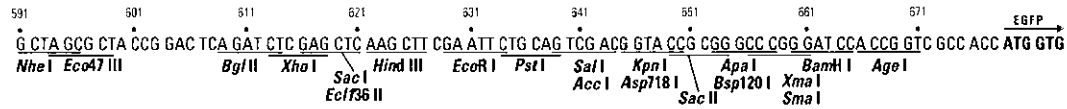
Name
2FYVE GFP
2FYVE RFP
GFP actin
pEF6 mcherry actin
AKTPH GFP
AKTPH RFP
AKT wt
Amphipathic helix GFP
Amphipathic helix Red
GFP-APPL1
pEGFPN3-Arf6 wt
pEGFPN3-Arf6 Q67L
pEGFPN3-Arf6 T27N
Arg K-Ras Red
Arp3 GFP
Bad GFP
Bad mCherry
Bax GFP
Bax-mCherry
Bcl2 mCherry
Bcl2 GFP
Bid GFP
Human Bid
Murine tBid
Bid^{G94E} GFP
$tBid^{G94E}$ GFP
$tBid^{G94E}$ GFP His
$tBid^{G94E}$ mCherry
Caspase 8
CD36-C
cdc42 DN GFP
CD63 GFP
pmCherry-C1
CKMT GFP
CKMT C-term GFP
CKMT GFP His 6
CKMT mCherry
CLBD GFP
CLBD mCherry
$CLBD^{+10}$ GFP His
$CLBD^{+10}$ GFP His6
Human Cox 7A1 MGC
Cyc1 GFP
Cyc1-mCherry
Human Cycl1 MGC 23492
GFP Crk2 wt
EEA1 myc
EEA1 Rab5 bd GFP
EK8+ GFP
Fc gamma R2a GFP
Fc gamma R2a RFP
Fc ERIG (Fc gamma)
FV FYVE GFP
FYVE dsRED2
eGFP C1
eGFP N1
GT46 GFP
HA-C1
HA-N1
IMS GFP
IMS mCherry
Kdel GFP
KR mRFP
Lifeact mRFP
Matrix GFP
Matrix mCherry
mito mRFP
EGFP-OCRL
OCRL mRuby
PH OSH 2 GFP
PA GFP C1
PA GFP N1
Pak1 (H83, 86L, K299R) myc
Pak1 PBD myc
Pak1 PBD YFP
Pak1 wt GFP
Pak1 wt myc
PBD YFP
GFP PDK1 wt
GFP PH(PDKdelta)
PI4K IIA GFP
PI4K IIB GFP
pIFP N1
pIfP C1
PIP5K CFP (IRAB)
PIPKIalpha YFP
PIPKIbeta YFP
PIPKIgamma GFP
PIPKIgamma KD GFP
PKCdelta (C1) GFP
2PH PLC GFP (tandem)
PLCdeltaPH GFP
PLCdeltaPH RFP
PM GFP
PM PA GFP
PM red
PM RFP
pcDNA3 PTEN C1245 A6 YFP
pcDNA3 PTEN wt YFP
Rab1 A
Rab1 B
Rabaptin5
Rab GAP5 GFP
Rab5 eGFP
Rab5a-eGFP
Rab5 DN GFP
Rab5 CA GFP
Rab7 DN myc
Rab7 GFP
Rab7Q67L YFP
Rab11 bp GST
wt Rab34 EGFP
Rac1 CA GFP
Rac1 DN GFP
Rac1 GFP wt
Rac2 GFP wt
Tail H-Ras GFP
Tail H-Ras RFP
Tail K pre RFP
Tail K-Ras GFP
Tail K-Ras Red
RFP-APPL1
DN RhoA GFP
RhoA CA GFP
RhoA wt GFP
pBabe RhoA Biosensor
pTriEX RhoA Biosensor
Rpre RFP
Syk GFP
Syk K369R GFP
Talin GFP
TC10 wt HA
GFP TC10 wt

Tom70-Chex-FKBP3
Dapi 2 / Tyro BP
eGFP-Vps11
eGFP-Vps16
eGFP-Vps18
eGFP-Vps33a
pEGFP VPS 34 wt
WAVE eGFP
WAVE2 wt GFP
Kdel GFP

Note: All plasmids are in the pEGFP vectors, or a variant thereof where the EGFP is replaced with another fluorescent protein.

GenBank Accession #U55762 Catalog #6085-1





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

Description:

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

Use:

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

Location of features:

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

Primer Locations:

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

Propagation in *E. coli*:

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

References:

1. Prasher, D. C. *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M. *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B. *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
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Info on Toxin(s)

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	10 µg
Proposed Storage Dose:	500 µg
LD $_{50}$ (species):	250 µg

Calculation:

250 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 12500

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 1250

Comments/Recommendations: