THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 ~~also~~ be updated at least every 3 years or when there are changes to the biohazards 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 Biohazard 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: Dr. Sean Cregan
SIGNATURE: [signature]
DEPARTMENT: Molecular Brain Research Group, RRI
ADDRESS: 100 Perth Drive, London, ON N6A 5K8
PHONE NUMBER: 519-663-5777 ext 24160
EMERGENCY PHONE NUMBER(S): 519-642-2758
EMAIL: scregan@robarts.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 3250

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: HSF / CIHR
GRANT TITLE(S): Mechanisms of Neuronal Apoptosis
BAX Activation in P53 Signalling in Oxidative damage induced Neuronal Apoptosis

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Meera Karajgikar
Jennifer Guadagno
Patrick Swan
Kristen Pitzul (Graduating in August 2009)
Rasha Shaikh (Graduating in August 2009)
Diana Steckley (On Leave)
Sarah Humphrey
Sarah Aubin
Kristin Ambacher
Cristina Silva (Post doctoral fellow Starting in September 2009)

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES O 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
E. coli DH5α	☒ Yes O No	O Yes O No	O Yes O No	500 mL	Invitrogen	O 1 ☒ 2 O 3
Adenovirus	☒ Yes O No	O Yes O No	O Yes O No	500 mL		O 1 ☒ 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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? ☒ 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	☒ Yes O No	Mouse Brain	2008-004-02
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in the table below. - See attachment 2.3

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be 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 No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
See attachment 4.2				

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
See attachment 4.3				

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ☒ NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus 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? ☒ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Mouse

6.3 AUS protocol # 2008-004-02

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

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____
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_____

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

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

10.5 What is your intention? 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

10.9 Please 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_____
If no, please proceed to Section 10.0 O NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 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 3

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

✓ YES, permit # if on-campus BIO-LL-0027
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: July 7/2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: July 09, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Description of the project

Our laboratory is investigating the molecular mechanisms that regulate apoptotic cell death in the affected nervous system. Both caspases and Caspase-independent death effectors such as Apoptosis Inducing Factor (AIF) contribute to the neuronal cell death response. Furthermore, activation of both of these pathways is regulated by the pro-apoptotic Bcl-2 family protein Bax. The laboratory is currently examining the role of P53 and other injury inducible factors in the regulation of Bax activation using in vitro and in vivo models of neuronal injury.

Dr. Cregan's laboratory is also studying the molecular signaling pathways involved in the regulation of apoptosis in neural stem cells. The discovery of stem cells within the brain has lead to much excitement as it is believed that these cells have the potential to regenerate damaged or diseased nervous tissue. However, the propensity of activated neural stem cells to undergo apoptosis has posed a major impediment to the success of such cell replacement therapies. Dr. Cregan's laboratory has developed a trophic factor deprivation model to study apoptosis in neural precursor cells. This research will lead to the identification of critical components of the apoptotic pathway in neural precursor cells which they will exploit to facilitate regeneration in the injured and diseased nervous system.

KEY RESEARCH ISSUES:

Define the molecular signaling pathways that regulate BAX activation and apoptosis following neuronal injury.

Identify the molecular processes involved in caspase-independent neuronal cell death.

Delineate the apoptotic signaling pathways in neural precursor cells.

Apoptosis is frequently triggered by events that alter the expression of key target genes. Under these circumstances, the genes involved can be identified by techniques that analyze gene expression.
Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to express particular genes of interest. Plasmids are also used to express proteins to pursue further studies.

Adenoviral vectors are used to deliver genetic material into cells. This process can be performed into neurons as it is not possible to use plasmids in to neurons. Protein coding genes can be expressed using viral vectors, commonly to study the function of the particular protein.

In order to study the interaction between genes of interest, use of stable cell lines is very important. Cos 7 cells are used to study co-localization of two or more proteins using Confocal Microscopy. HEK 293 cells are used to study effect of different treatments before we proceed with primary cultures. SH-SY5Y and Neuro-2A cells are used as these are neuronal cell lines and it is more relevant to our laboratory's focus. To manipulate cells involves the introduction of foreign DNA by transfection. This is often performed to cause cells to

express a protein of interest. More recently, the transfection of Si-RNA constructs have been realized as a convenient mechanism for suppressing the expression of a particular gene/protein.

Attachment 2-3

Biosafety-section 2.3		
Cell Type	**Specific Cell line**	**Supplier/Source**
Human	HEK-293	Rylett Lab
Human	SH-SY5Y	Rylett Lab
Rhodent	Neuro-2A	Strong Lab
Non-Human Primate	COS-7	Ferguson Lab

Attachment 4.2

Bacteria used for cloning	Plasmid:	Plasmid	Source of plasmid	Gene Transfected	Change
E.coli	pEGFP GFP BAX	pEGFP	Clontech	Bax	No Change
E.coli	pcDNA3 FLAG - E2F1 WT	pCDNA-3	Invitrogen	E2F1	No Change
E.coli	pBS SK II (+) BIM L	pEGFP-C1	Clontech	Bim-L	No Change
E.coli	pCEP Puma Ha	pCEP-4	Invitrogen	PUMA	No Change
E.coli	pRC - Bcl2 acta	pRc/CMV	Invitrogen	Bcl2 Acta	No Change
E.coli	pRC/CMV Bcl2 CB5 (664)	pRc/CMV	Invitrogen	Bcl2 CB5	No Change
E.coli	PCDNA3 (+) Mcl - I Flag	pCDNA-3.1	Invitrogen	Mcl-I	No Change
E.coli	TP53INP1 (alpha) - pCDNA4	pCDNA-4	Invitrogen	TP-53-INP-I Alpha	No Change
E.coli	TP53INP1β - pCDNA4	pCDNA-4	Invitrogen	TP-53-INP-I Beta	No Change
E.coli	TP53INP1 (alpha) - pEGFP	pEGFP-C1	Clontech	TP-53-INP-I Alpha	No Change
E.coli	TP53INP1β - pEGFP	pEGFP-C1	Clontech	TP-53-INP-I Beta	No Change
E.coli	Lamp-I GFP	pEGFP-C1	Clontech	LAMP-I	No Change
E.coli	Bcl-XL in GFP	pEGFP-C1	Clontech	Bcl-XL	No Change
E.coli	Bcl-2 in GFP	pEGFP-C1	Clontech	Bcl-2	No Change
E.coli	p3X Flag-NOXA	p3X-Flag-Myc-CMV	Sigma	NOXA	No Change
E.coli	EGR-1 in 3X-Flag	p3X-Flag-Myc-CMV	Sigma	EGR-I	No Change
E.coli	SP1 in pCDNA3	pCDNA-3	Invitrogen	SP-I	No Change
E.coli	CHOP-in GFP	pEGFP-C1	Clontech	CHOP	No Change
E.coli	ATF-4 in GFP	pEGFP-C1	Clontech	ATF-4	No Change
E.coli	GSK3-beta in pCDNA3	pCDNA-3	Invitrogen	GSK-3-Beta	No Change
E.coli	FOXO3-CA in pCDNA3	pCDNA-3	Invitrogen	FOXO-3CA	No Change

Attachment 4.3.

Biosafety Section 4.3

Virus used for Transfection	Vector	Source of Vector	Gene Transfected	Change that results
Adenovirus	pAd-lox-GFP	David Park Lab	None	No change
Adenovirus	pAd-lox	David Park Lab	CDK-4	No change
Adenovirus	pAd-lox	David Park Lab	p-53	No change
Adenovirus	pAd-lox	David Park Lab	CDK-4 DN	No change
Adenovirus	pADTrack	David Park Lab	GSK-3-Beta	No change
Adenovirus	pADTrack	David Park Lab	GSK-3-Beta-CA	No change
Adenovirus	pADTrack	David Park Lab	FOXO-3CA	No change

None of the adenoviruses will be injected into live animals (mice).

Hsuan Chen

Ron Noseworthy

From: Sean Cregan [scregan@robarts.ca]
Sent: July 9, 2009 11:30 AM
To: Ron Noseworthy
Subject: biohazard registry

Hi Ron,

This message is to confirm that we will not be injecting any adenoviruses into live animals (mice).

Regards,

Sean Cregan

Subject: Fw: Bio Form
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Mon, 13 Jul 2009 14:51:43 -0400
To: jstanle2@uwo.ca

----- Original Message -----
From: Ron Noseworthy
To: 'scregan@robarts.ca' <scregan@robarts.ca>
Sent: Mon Jul 13 14:46:00 2009
Subject: Bio Form

Hi Dr Cregan

Can you tell me where Dr David Park is located and have you ever imported any material?

Ron

***
This message is intended only for the individual or entity to which it is addressed and may contain information that is privileged, confidential and exempt from disclosure under applicable law. If you are not the intended recipient, any dissemination, distribution or copying of this communication is prohibited. If you have received this communication in error, please notify the sender and delete the original. Thank you.

Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$264.00**
Designations:	COS-7	Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent fibroblast
Organism:	*Cercopithecus aethiops*	Morphology:	PHOTO
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.		

Related Cell Culture Products

Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region
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.
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
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach

may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

1822: Gluzman Y. SV40-transformed simian cells support the replication of early SV40 mutants. Cell 23: 175-182, 1981. PubMed: 6260373
32447: Fernandez LM, Puett D. Lys583 in the third extracellular loop of the lutropin/choriogonadotropin receptor is critical for signaling. J. Biol. Chem. 271: 925-930, 1996. PubMed: 8557706
32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614
32500: Campbell M, et al. The simian foamy virus type 1 transcriptional transactivator (Tas) binds and activates an enhancer element in the gag gene. J. Virol. 70: 6847-6855, 1996. PubMed: 8794326
32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. J. Virol. 70: 7921-7928, 1996. PubMed: 8892915
32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. J. Biol. Chem. 271: 24105-24114, 1996. PubMed: 8798649
32566: Dittrich E, et al. A di-leucine motif and an upstream serine in the interleukin-6 (IL-6) signal transducer gp130 mediate ligand-induced endocytosis and down-regulation of the IL-6 receptor. J. Biol. Chem. 271: 5487-5494, 1996. PubMed: 8621406
32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812
32720: Chen Y, et al. Demonstration of binding of dengue virus envelope protein to target cells. J. Virol. 70: 8765-8772, 1996. PubMed: 8971005
32728: Russell DW, Miller AD. Foamy virus vectors. J. Virol. 70: 217-222, 1996. PubMed: 8523528
32861: Wright DA, et al. Association of human fas (CD95) with a ubiquitin-conjugating enzyme (UBC-FAP). J. Biol. Chem. 49: 31037-31043, 1996. PubMed: 8940097
32893: Zhang J, et al. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J. Biol. Chem. 271: 18302-18305, 1996.

Cell Biology

ATCC® Number:	**CCL-131™** Order this Item	Price:	**$256.00**
Designations:	Neuro-2a	Depositors:	RJ Klebe
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)	Morphology:	neuronal and amoeboid stem cells 

Source:	**Strain:** A **Organ:** brain **Disease:** neuroblastoma **Cell Type:** neuroblast;
Cellular Products:	acetylcholinesterase tubulin
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.

Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Herpes simplex virus Vesicular stomatitis virus Human poliovirus 1
Reverse Transcript:	negative
Antigen Expression:	H-2, a haplotype; Mus musculus, expressed
Cytogenetic Analysis:	modal number = 95; range = 59 to 193. Karyotype unstable within a stemline range of 94 to 98 chromosomes. All the cells contain 6 to 10 large chromosomes with median or submedian centromeres and 2 to 4 minute chromosomes.
Geno Type:	albino
Comments:	Clone Neuro-2a was established by R.J. Klebe and F.H. Ruddle from a spontaneous tumor of a strain A albino mouse. This tumor line, designated C1300, was obtained from the Jackson Laboratory, Bar Harbor, Maine [22161].Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells.The cell line has been used for studies on the mechanism of vinblastine precipitation of microtubular protein, the kinetics of GTP binding to isolated protein, the turnover of microtubules in vivo, and the synthesis and assembly of microtubular protein [PubMed: 5263744]. The World Organization for Animal Health (OIE) uses the cells for routine diagnosis of rabies. (see: http://www.oie.int/Eng/Normes/Mmanual/A_00044.htm)Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. 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 **Protocol:**

1. Remove and discard culture medium.

Subculturing:

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

Preservation:

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 1 to 2 times per week

Freeze medium: Complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

1023: Olmsted JB, et al. Isolation of microtubule protein from cultured mouse neuroblastoma cells. Proc. Natl. Acad. Sci. USA 65: 129-136, 1970. PubMed: 5263744
22161: Klebe RJ, Ruddle FH. Neuroblastoma: Cell culture analysis of a differentiating stem cell system. J. Cell Biol. 43: 69A, 1969.
29352: Naslavsky N, et al. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J. Biol. Chem. 272: 6324-6331, 1997. PubMed: 9045652
29861: Kaneko K, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94: 10069-10074, 1997. PubMed: 9294164
32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

Return to Top

Cell Biology

ATCC® Number:	**CRL-2266™** Order this Item	Price:	**$264.00**
Designations:	SH-SY5Y	Depositors:	JL Biedler
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	mixed, adherent and suspension
Organism:	*Homo sapiens* (human)	Morphology:	epithelial 

Source:
Organ: brain
Disease: neuroblastoma
Derived from metastatic site: bone marrow

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.

Related Cell Culture Products

Restrictions: NOTE: SH-SY5Y was deposited at the ATCC by June L. Biedler, Memorial Sloan-Kettering Cancer Center. SH-SY5Y is distributed for academic research purposes only. Memorial Sloan-Kettering releases the line subject to the following: 1.) SH-SY5Y or its products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of SH-SY5Y including any use by a for-profit entity must first be negotiated with Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1970

Applications: transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)

Antigen Expression: Blood Type A; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 11
D13S317: 11
D16S539: 8,13
D5S818: 12
D7S820: 7,10
THO1: 7,10
TPOX: 8,11
vWA: 14,18

Cytogenetic Analysis: modal number = 47; the cells possess a unique marker comprised of a chromosome 1 with a complex insertion of an additional copy of a 1q segment into the long arm, resulting in trisomy of 1q [22554]

Age: 4 years

Gender: female

Comments: SH-SY5Y cells have a reported saturation density greater than 1 X 10(6) cells/sq cm. They are reported to exhibit moderate levels of dopamine beta hydroxylase activity [PubMed ID: 29704].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is a 1:1 mixture of ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003, and F12 Medium. 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
Subculturing:	**Protocol:** These cells grow as a mixture of floating and adherent cells. The cells grow as clusters of neuroblastic cells with multiple, short, fine cell processes (neurites). Cells will aggregate, form clumps and float. Remove the medium with the floating cells, and recover the cells by centrifugation. Rinse the adherent cells with fresh 0.25% trypsin, 0.53 mM EDTA solution, add an additional 1 to 2 ml of trypsin solution, and let the culture sit at room temperature (or at 37C) until the cells detach. Add fresh medium, aspirate, combine with the floating cells recovered above and dispense into new flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:20 to 1:50 is recommended **Medium Renewal:** Every 4 to 7 days
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	parental cell line:ATCC HTB-11 recommended serum:ATCC 30-2020
References:	22554: Ross RA, et al. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. J. Natl. Cancer Inst. 71: 741-749, 1983. PubMed: 6137586 23032: Biedler JL, et al. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 38: 3751-3757, 1978. PubMed: 29704

Return to Top

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 epithelial
Organism:	*Homo sapiens* (human)	Morphology:	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.

Related Cell Culture Products

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:
This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age:
fetus

Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.

Comments:

The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. 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
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

derivative: ATCC CRL-12006
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852

21624: Xie QW, et al. Complementation analysis of mutants of nitric oxide synthase reveals that the active site requires two hemes. Proc. Natl. Acad. Sci.

MATERIAL SAFETY DATA SHEET

Revised 9/03/02
Replaces 6/19/02
Printed 9/10/02

MAX EFFICIENCY DH5ALPHAF'IQ COMPETENT CELLS
INVITROGEN CORPORATION
MSDS ID: 18288

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

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

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name:
MAX EFFICIENCY DH5ALPHAF'IQ COMPETENT CELLS

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therpeutic, or other clinical uses.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
DIMETHYL SULFOXIDE	67-68-5	3 - 7
GLYCEROL	56-81-5	7 - 13

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if absorbed.
****

Potential Health Effects:
Eye:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin:
Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage.
Upon prolonged or repeated exposure, harmful if absorbed through the skin. May cause minor systemic damage.

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.
No toxicity expected from inhalation.

Ingestion:
Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin:
Wash with soap and water. Get medical attention if irritation develops or persists.

Inhalation:
Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion:
Do not induce vomiting and seek medical attention immediately. Drink two

4. FIRST AID MEASURES (CONT.)

glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C: Not available.

Upper Flammable Limit %: Not available.

Lower Flammable Limit %: Not available.

Autoignition Temperature Deg C: Not available.

Extinguishing Media:
Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. Use water spray/fog for cooling.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Includes carbon dioxide, carbon monoxide, dense smoke.

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider

6. ACCIDENTAL RELEASE MEASURES (CONT.)

the expertise of employees in the area responding to the spill.
Ventilate the contaminated area.
Prevent the spread of any spill to minimize harm to human health and the environment if safe to do so. Wear complete and proper personal protective equipment following the recommendation of Section VIII at a minimum. Dike with suitable absorbent material like granulated clay. Gather and store in a sealed container pending a waste disposal evaluation.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
Keep closed or covered when not in use.

Storage Procedures:
Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
DIMETHYL SULFOXIDE	Not established.	Not established.
GLYCEROL	15	10 MG/M3

Engineering Controls:
Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
An eye wash station must be available where this product is used.
Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Have a safety shower available.

Respiratory:
Use supplied-air respiratory equipment as required.
NIOSH approved air purifying respirator with dust/mist filter.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
Boiling Point (C):	Not established.
Melting Point (C):	Not established.
Solubility in water:	Not established.
pH:	Not established.
Vapor Pressure:	Not established.
Vapor Density:	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalies. Temperatures above flash point in combination with sparks, open flames, or other sources of ignition.

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Sulfur containing gases.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG
Glycerol: 12600 MG/KG

Target Organs: Blood. Eyes. Skin. Kidneys.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades quickly.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Hazard Class:
Subsidiary Hazards:
ID Number:
Packing Group:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
DIMETHYL SULFOXIDE	D2B
GLYCEROL	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES: None assigned.

PRODUCT SAFETY PHRASES: None assigned.

PRODUCT CLASSIFICATION: Not classified.

Component	EINECS Number
DIMETHYL SULFOXIDE	200-664-3
GLYCEROL	200-289-5

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

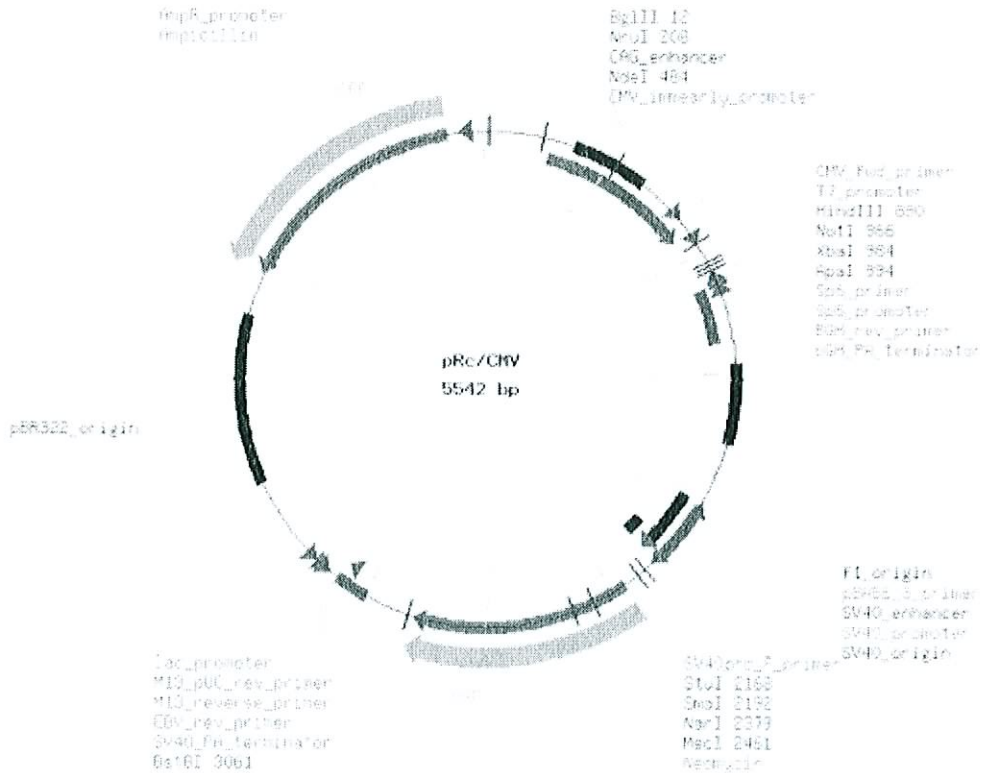
Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

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 Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

Vector Backbone: pRc/CMV

Vendor:	Invitrogen
Alternate Vector Names:	RcCMV
Vector Type:	Mammalian
Promoter:	CMV
Backbone Size (bp):	5542
Sequencing Primer:	T7/Sp6
Bacteria Resistance:	Amp
Mammalian Selection:	Neomycin
Catalog Number:	V75020
Sequence and Map:	Sequence (Click to see features and cutters)

Click on map to enlarge



SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.1
Revision Date 06/18/2009
Print Date 07/02/2009

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : p3xFLAG-Myc-CMV™-23 Expression Vector

Product Number : E6026
Brand : Sigma

Company : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA
Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # : 800-424-9300

2. COMPOSITION/INFORMATION ON INGREDIENTS

CAS-No.	EC-No.	Index-No.	Concentration
Water			
7732-18-5	231-791-2	-	99.7558 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	0.157 %
Ethylenediaminetetraacetic acid disodium dihydrate			
6381-92-6	205-358-3	-	0.0372 %
Deoxyribonucleic acids, plasmid ColE1			
100209-25-4	309-333-9	-	0.05 %

3. HAZARDS IDENTIFICATION

WHMIS Classification

Not WHMIS controlled. Not WHMIS controlled.

HMIS Classification

Health Hazard: 0
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

4. FIRST AID MEASURES

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING MEASURES

Flammable properties

Flash point	no data available
Ignition temperature	no data available

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid dust formation.

Environmental precautions
No special environmental precautions required.

Methods for cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Handling
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
For prolonged or repeated contact use protective gloves.

Eye protection
Safety glasses

Hygiene measures
General industrial hygiene practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Storage stability
Stable under recommended storage conditions.

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

no data available

Irritation and corrosion

no data available

Sensitisation

no data available

Chronic exposure

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

12. ECOLOGICAL INFORMATION

Elimination information (persistence and degradability)

no data available

Ecotoxicity effects

no data available

Further information on ecology

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Deoxyribonucleic acids, plasmid ColE1	100209-25-4

WHMIS Classification

Not WHMIS controlled.	Not WHMIS controlled.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

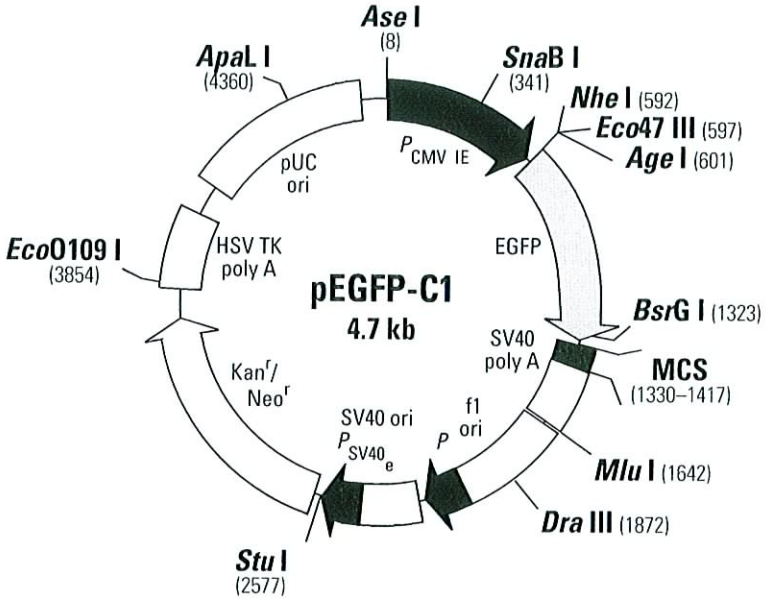
Vector Information

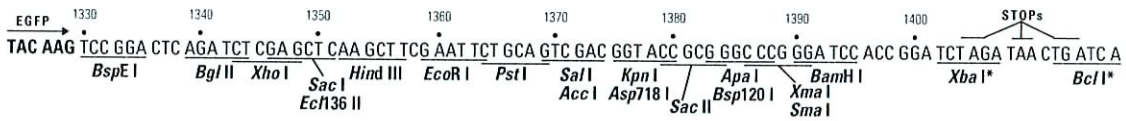
pEGFP-C1 Vector Information

PT3028-5

GenBank Accession #: U55763

Catalog #6084-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. All restriction sites shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pEGFP-C1 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-C1 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-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-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 (Neor), 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-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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Use

Fusions to the C 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-C1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. 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-C1 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
- Enhanced green fluorescent protein gene
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615; Stop codon: 1408–1410
  - Insertion of Val at position 2: 616–618
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  - His-231 to Leu mutation (A→T): 1307
  - Last amino acid in wild-type GFP: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2164–2169; –10 region: 2187–2192
  - Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  - 21-bp repeats: 2423–2443, 2444–2464, & 2466–2486
  - Early promoter element: 2499–2505
  - Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2627–2629; stop codon: 3419–3421
  - G→A mutation to remove *Pst* I site: 2809
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 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.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

invitrogen

Material Safety Data Sheet

Revision Date: 26-Mar-2009

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

Product code 350717
Product name PCDNA3.1/MYC-HIS A 20UG

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

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

Form
Solid

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	No information available

3. HAZARDS IDENTIFICATION

Inhalation May cause irritation of respiratory tract.
Ingestion No information available

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

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

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.

Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Solid

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	May cause eye irritation with susceptible persons.
Skin	No information available
Inhalation	May cause irritation of respiratory tract.
Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

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

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

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End of Safety Data Sheet