The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Dr. Sean Cregan**
DEPARTMENT:	**Molecular Brain Research Group, Robarts Research**
ADDRESS:	**Rm 3250, 100 Perth Drive London ON N6A 5K8**
PHONE NUMBER:	**519-663-5777 Ext. Lab 24160 Office: 24134**
EMERGENCY PHONE NUMBER(S):	**Home: 519-642-2758, Cell: 226-448-2758**
EMAIL:	**scregan@robarts.ca**

Location of experimental work to be carried out :

Building : **Robarts research Institute** Room(s): **3250**

Building : _____ Room(s): _____

Building : _____ Room(s): _____

***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: **HSF/CIHR**

GRANT TITLE(S): **Mechanisms of Bax activation in Neuronal Apoptosis, P53 Signaling in Oxidative Damage Induced Neuronal Apoptosis**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Meera Karajgikar	**mkarajgi@uwo.ca**	**April 2004**
Jennifer Guadagno	**jguadag2@uwo.ca**	**January 2010**
Patrick Swan	**pswan@uwo.ca**	**January 2006**
Kristin Ambacher	**kambache@uwo.ca**	**October 2008**
Lisa Foris	**lforis@uwo.ca**	**February 2012**

Elizabeth Merwin	emerwin@uwo.ca	August 2010
Lee-Anne Fochesato	lfochesa@uwo.ca	Sept 2011

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

All hazardous material are labeled properly. Refrigerators/freezers are cleaned and defrosted on a regular basis. All plasticware and glassware are autoclaved. Dead animals are incinerated

Bacteria:
The E.coli bacteria is used in our laboratory to generate plasmid DNA to study function and cell localization of genes of interest. The glycerol stock of bacteria is kept in a -80C freezer whereas the bacterial cultures are kept in the fridge. Proper personal protective equipment is worn at all times when handling the bacterial cultures. Once the experimental work has been completed, all the glassware is disinfected with freshly prepared 10% bleach, rinsed with plenty of water and autoclaved.

Adenoviruses:
An adenoviral system is used in our laboratory to generate adenoviruses to study function and cell localization of genes of interest in primary neuronal cells.
All the adenoviral work is done in a bio-safety cabinet that is cleaned with viruside(Clidox or Quatricide), then with 70% ethanol and air dried following each use of the virus.
All serological pipettes, pipette tips are decontaminated in a virucide(Clidox or Quatricide) for at least 30 minutes prior to discarding into biohazard waste container.
All solid waste material related to the adenoviral experiments are placed in biohazard waste bag and sealed for disposal.
To prevent cross contamination, we have a separate set of pipettes, and only barrier tips are used. Also we have a separate incubator to store cells infected with adenoviruses.

Changes on this page

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

See Attachment 1

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species ____________________
What is the origin of the microorganism(s)? ____________________

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific 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 DH5Alpha	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**500ml**	**invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Adenovirus	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☒ No	**500ml**	**Vector Biolabs**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: **See Attachment 2**

Changes made to 1.2

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

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

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

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	☒ Yes ☐ No	**HEK 293**	**II**	**ATCC**
Rodent	☒ Yes ☐ No	**SH-SY5Y, N2A**	**II**	**ATCC**
Non-human primate	☒ Yes ☐ No	**COS-7**	**II**	**ATCC**
Other (specify)	☐ Yes ☐ 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 ☐ 1 ☒ 2 ☐ 2+ ☐ 3

Additional Comments: **See Attachment 3**

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments:

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning * E. coli	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
DH5-alpha	**See Attachment 4**		**See Attachment 4**	**Contains an antibiotic resistant plasmid**	**No**	**None**

** Please attach a Material Safety Data Sheet or equivalent if available.*
*** Please attach a plasmid map.*
****No Material Safety Data Sheet is required for the following strains of E. coli:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☒ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Adenovirus	**See Attachment 2**	**Vector Biolabs**	**See attachment 2**	**See attachment 2**

** Please attach a Material Safety Data Sheet or equivalent.*

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☒ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: **Attachment 4**

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Mouse**

7.3 AUS protocol # **2008-004-02**

7.4 List the location(s) for the animal experimentation and housing. **ACVS Rm 5543, RRI Room # 3241, 3250**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☒ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☒ NO

8.3 If **YES**, please specify the animal(s) used:

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

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

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

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

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

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

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

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

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

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

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 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.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Dr. Sean Cregan ***Date:*** April 18,2012

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: April 09, 2012.
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **Bio-41-0027**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposur
needlestick injury or an accidental splash:

SEE E-mail

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Dr. Sean Cregan ***Date:*** April 18,2012

15.4 Additional Comments: _____

16.0 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: May 29, 2012

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

Special Conditions of Approval:

CREGAN LABORATORY GUIDELINES FOR THE SAFE HANDLING OF ADENOVIRAL VECTORS

ADENOVIRAL EXPERIMENTS --- Standard Operating Procedures:

- Laboratory coats, gloves and safety glasses are worn while handling the adenoviral vectors.

- Adenoviral containing materials are handled inside the biological safety cabinet (BSC) in room 3251.

- All tissue culture work related to adenoviral vectors experiments are conducted inside the BSC.

    - Only materials needed for adenoviral experiments are placed in BSC.

    - All serological pipettes, pipette tips are decontaminated in a virucide (Clidox, Quatricide or freshly prepared 10% household bleach) for 30 minutes prior to discarding into biohazard waste container.

    - Upon completion of work inside the BSC, all work surfaces and equipment used inside the BSC are sprayed with the virucide (Clidox or Quatricide) and then with 70% ethanol and air-dried.

    - All solid waste materials related to the adenoviral experiments are placed in biohazard waste bag and sealed for disposal (i.e. to be autoclaved).

    - Vacuum lines for liquid waste collection are filtered with a HEPA filtered before entering the vacuum system. For aspirated liquid waste, aspirate full-strength bleach through the suction tube into the liquid waste container to the approximate final concentration (1 in 10) and soak for 20-30 minutes and empty entire contents down the drain. Rinse drain and liquid waste flask with 70% ethanol.

- Transportation of adenoviral vector containing materials will be done in plastic containers (50 ml conical tubes) contained inside a leak-proof container.

- All the centrifugation containing adenoviruses is done in swinging bucket rotor which is sealed with aerosol tight screw caps.

In case of adenoviral vector spill outside the BSC, warn everyone in the immediate area contain spill with bleach soaked paper towels and mop spill with paper towels, re-apply bleach and soak for 30 minutes. All waste materials are placed in biohazard bag and area is cleaned again with bleach solution followed by 70% ethanol wash.

Subject: Cregan BHARF
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: 6/4/2012 8:45 AM
To: "jstanle2@uwo.ca" <jstanle2@uwo.ca>

-----Original Message-----
From: mkarajgikar [mailto:mkarajgikar@robarts.ca]
Sent: June-01-12 8:09 AM
To: Ron Noseworthy
Subject: Biosafety 2012

Hi Ron,

Here is the explanation for 15.2 in the biosafety renewal form:

In case of needlestick injury or an accidental splash; the worker should wash the exposed site with soap and water immediately. He must inform the supervisor of the incident. He must seek prompt medical attention at workplace health during the hours of operation or the nearest hospital emergency departmrnt or emergency clinic.

I apologize for missing this important information.

Meera Karajgikar

Attachment 1

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 (modified).

Virus used for Transduction	Vector	Source of Vector	Gene Transfected	Change that results
Adenovirus	pAd-lox-GFP	Cell Biolabs	GFP	Express GFP
Adenovirus	pAd-lox	Cregan Lab	CDK-4	Express CDK-4
Adenovirus	pAd-lox	Cregan Lab	p-53	Express P-53
Adenovirus	pAd-lox	Cregan Lab	CDK-4 DN	Express CDK-4 DN
Adenovirus	pADTrack	Cregan Lab	GSK-3-Beta	Express GSK-3-Beta
Adenovirus	pADTrack	Cregan Lab	GSK-3-Beta-CA	Express GSK-3-Beta-CA
Adenovirus	pADTrack	Cregan Lab	FOXO-3CA	Express FOXO3-CA
Adenovirus	pADTrack	Cregan Lab	Noxa	Express NOXA
Adenovirus	Adlox	Cregan Lab	Rb-WT-IRES-GFP	Express GFP
Adenovirus	Adlox	Cregan Lab	p53(22/23-53/54	Express P-53
Adenovirus	Adlox	Cregan Lab	HA-PUMA	Express PUMA
Adenovirus	Adlox	Cregan Lab	p53-173L	Express P-53
Adenovirus	Adlox	Cregan Lab	dp1-WT	Express GFP
Adenovirus	Adlox	Cregan Lab	p16	Express P-16
Adenovirus	Adlox	Vector Biolabs	Cre-GFP	Express GFP-Cre
Adenovirus	Adlox	Cregan Lab	ΔNp 73	Express DN P73
Adenovirus	Adlox	Cregan Lab	dn CDK6	Express DN-CDK-6
Adenovirus	Adlox	Cregan Lab	Flag-Mcl1	Express FLAG-MCL1
Adenovirus	Adlox	Cregan Lab	dn-CDK2	Express DN-CDK-2
Adenovirus	Adlox	Cregan Lab	CDK6	Express CDK-6

Note: All these adenoviruses are used to study functioning and cell localization of the genes of interest.
None of these viruses are used for cell transformations.

Virus used for Transduction	Vector	Source of Vector	Gene Transfected	Change that results
Adenovirus	pAd-lox-GFP	Cell Biolabs	GFP	Express GFP
Adenovirus	pAd-lox	David Park Lab	CDK-4	Express CDK-4
Adenovirus	pAd-lox	David Park Lab	p-53	Express P-53
Adenovirus	pAd-lox	David Park Lab	CDK-4 DN	Express CDK-4 DN
Adenovirus	pADTrack	David Park Lab	GSK-3-Beta	Express GSK-3-Beta
Adenovirus	pADTrack	David Park Lab	GSK-3-Beta-CA	Express GSK-3-Beta-CA
Adenovirus	pADTrack	David Park Lab	FOXO-3CA	Express FOXO3-CA
Adenovirus	pADTrack	David Park Lab	Noxa	Express NOXA
Adenovirus	Adlox	David Park Lab	Rb-WT-IRES-GFP	Express GFP
Adenovirus	Adlox	Cregan Lab	p53(22/23-53/54	Express P-53
Adenovirus	Adlox	Cregan Lab	HA-PUMA	Express PUMA
Adenovirus	Adlox	Cregan Lab	p53-173L	Express P-53
Adenovirus	Adlox	Cregan Lab	dp1-WT	Express GFP
Adenovirus	Adlox	Cregan Lab	p16	Express P-16
Adenovirus	Adlox	Vector Biolabs	Cre-GFP	Express GFP-Cre
Adenovirus	Adlox	Cregan Lab	ΔNp 73	Express DN P73
Adenovirus	Adlox	Cregan Lab	dn CDK6	Express DN-CDK-6
Adenovirus	Adlox	Cregan Lab	Flag-Mcl1	Express FLAG-MCL1
Adenovirus	Adlox	Cregan Lab	dn-CDK2	Express DN-CDK-2
Adenovirus	Adlox	Cregan Lab	CDK6	Express CDK-6

GFP Recombinant Adenovirus

CATALOG NUMBER: ADV-004 **STORAGE:** -80°C

QUANTITY AND CONCENTRATION: 50 µl, 1 x 10^{11} VP/mL in TBS containing 10% Glycerol

Background

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages in using an adenovirus to introduce genetic material into host cells. The permissive host cell range is very wide. The virus has been used to infect many mammalian cell types (both replicative and non-replicative) for high expression of the recombinant protein. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with liposome. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells.

Safety Consideration

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. Always wear gloves, use filtered tips and work under a biosafety hood.

Methods

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. If not enough virus is used, it will not give 100% of infection. If too much virus is used, it will cause cytotoxicity or other undesired effects. The amount of adenovirus cell surface receptors vary greatly among different cell types therefore the optimal concentration differs dramatically between cell types. A range of 10-200 MOI (multiplicity of infection) is used for most cell lines, but up to 1000 MOI may be used for lymphoid cell lines.

Traditionally, Infectivity particles are measured in culture by a plaque-forming unit assay (PFU) that scores the number of viral plaques as a function of dilution. In contrast to the 10-day infection of a classical plaque assay, Cell Biolabs' QuickTiter™ Adenovirus Titer Immunoassay Kit (Cat. #VPK-109) only requires 2-day infection, and there is no agar overlay step. The kit antibody against hexon protein recognizes all serotypes of adenovirus by immunocytochemistry (see Flow Chart).

Seed 293 cells in 24 or 12-well plate for 1 hr

↓

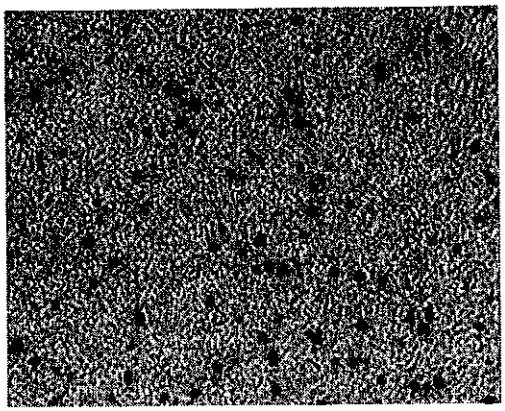
Prepare Adenovirus Serial Dilutions and Infect 293 cells for 48 hrs

↓

Anti-Hexon Immunocytochemistry Staining

↓

Count Positive Cells and Calculate Viral Titer



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3. Huang, S., Stupack, D., Mathias, P., Wang, Y., and Nemerow, G. (1997) *Proc. Natl. Acad. Sci. U S A.* 94, 8156-8161.
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Recent Product Citations

1. Perez-Moreno, M. et al. (2008). Loss of p120 catenin and links to mitotic alterations, inflammation, and skin cancer. *Proc. Natl. Acad. Sci. U S A* **105**:15399-15404.
2. Black, S.A. et al. (2008). TGFß1 stimulates connective tissue growth factor (CCN2/CTGF) expression in human gingival fibroblasts through a RhoA-independent, Rac1/Cdc42-dependent mechanism: statins with forskolin block TGFß1-induced CCN2/CTGF expression. *J. Biol. Chem.* **283**:10835-10847.
3. Jones, S.W. et al. (2009). Mitogen-activated protein kinase-activated protein kinase (MK2) modulates key biological pathways associated with OA disease pathology. *Osteoarthritis and Cartilage* **17**:124-131.

Warranty

Contact Information

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USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Material Safety Data Sheet

PRODUCT IDENTIFICATION

Catalog Number: ADV-xxx
Product Name: All Recombinant Adenoviruses

MANUFACTURER:

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126

EMERGENCY CONTACT:

+1 858 271 6500
info@cellbiolabs.com

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Cell Biolabs.
BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a

physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.
OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Cell Biolabs, Inc assumes no liability resulting from use of the above products.

7758 Arjons Drive, San Diego, CA 92126
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info@cellbiolabs.com

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

User Manual for Ready-To-Use Recombinant Adenovirus

CONTENTS AND STORAGE

Recombinant adenovirus is supplied in liquid form at indicated titer. The storage solution is DMEM/2.5% glycerol. Store at -80°C. If desired, aliquot viral stock upon arrival, and store those aliquots at -80°C freezer immediately. ***DO NOT FREEZE AND THAW REPEATEDLY.***

DESCRIPTION

Recombinant adenovirus is for delivering interested genes into mammalian cells. It provides the following advantages:

(1) 100% efficiency of gene delivery in many cell types.
(2) Recombinant viruses can be added directly to cells in culture medium (in the presence or absence of serum).
(3) It is not necessary to remove viruses, change or add medium following infection, although viruses can be removed after 6-12 hours post infections.

IMPORTANT GUIDELINES

Follow these guidelines when performing infections:

1. **Prepare virus-containing media:**
Thaw viral stock at either room temperature or on ice.
Add desired amount of virus to media. If needed, viruses could be diluted further in DMEM or other media

2. **Infecting cells with virus:**
Remove the original cell culture media, and add the above virus-containing media to cell culture. Below is a general guideline for the amount of media used:

24-well plate:	0.2-0.3 ml
12-well plate:	0.5-0.8 ml
6-well plate:	1-1.5 ml/well
60mm-plate:	3-4 ml/plate
10cm-plate:	8-12 ml/plate

Incubate cells with the virus-containing media for 6-12 hours, or as long as you wish.
(Optional), you could remove virus-containing media and replace it with fresh, desired media.

The appropriate amount of viruses used for infecting cells is critical for the outcome of your experiments. The goal is to get 100% of infection without causing cytotoxicity or other undesired effects. The amount of adenovirus cell surface receptors vary greatly among different cell types therefore the optimal concentration differs dramatically between cell types. A range of 10-100 MOI (multiplicity of infection) is used for most cell lines, but up to 1000 MOI may be used for lymphoid cell lines.

To determine this optimal concentration of virus for your study, you could conduct pilot testing in your cell line by using reporter adenoviruses, such as ß-gal adenovirus (Cat.#1080) or GFP adenovirus (Cat.#1060).

Date of revision: Jan. 30, 2005

3701 Market Street, Suite 340, Philadelphia, PA 19104 Tel: 877-biolabs Fax: 215-525-1112 info@vectorbiolabs.com

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045
http://www.vectorbiolabs.com

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

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Attachment 3

Attachment 3

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:

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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
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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 PHOTO

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

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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

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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.

Attachment 4. (modified)

Bacteria used	Plasmid:	Plasmid	Source of plasmid	Gene Transfected	Change
E.coli	pEGFP GFP BAX	pEGFP	Clontech	Bax	Express GFP-Bax protein
E.coli	pcDNA3 FLAG - E2F1 WT	pCDNA-3	Invitrogen	E2F1	Express Flag-E2F1
E.coli	pBS SK II (+) BIM L	pEGFP-C1	Clontech	Bim-L	No Change
E.coli	pCEP Puma Ha	pCEP-4	Invitrogen	PUMA	Express HA-PUMA
E.coli	pRC - Bcl2 acta	pRc/CMV	Invitrogen	Bcl2 Acta	Express BCl2-Acta
E.coli	pRC/CMV Bcl2 CB5 (664)	pRc/CMV	Invitrogen	Bcl2 CB5	Express Bcl2-CB5
E.coli	PCDNA3 (+) Mcl - I Flag	pCDNA-3.1	Invitrogen	Mcl-I	Express Flag-MCl-1
E.coli	TP53INP1 (alpha) - pCDNA4	pCDNA-4	Invitrogen	TP-53-INP-I Alpha	Express TP53INP1(Alpha)
E.coli	TP53INP1β - pCDNA4	pCDNA-4	Invitrogen	TP-53-INP-I Beta	Express TP53INP1(Beta)
E.coli	TP53INP1 (alpha) - pEGFP	pEGFP-C1	Clontech	TP-53-INP-I Alpha	Express GFP-TP53INP1 Alpha
E.coli	TP53INP1β - pEGFP	pEGFP-C1	Clontech	TP-53-INP-I Beta	Express GFP-TP53INP1 Beta
E.coli	Lamp-I GFP	pEGFP-C1	Clontech	LAMP-I	Express GFp-LAMP1
E.coli	Bcl-XL in GFP	pEGFP-C1	Clontech	Bcl-XL	Express GFP-Bcl-XL
E.coli	Bcl-2 in GFP	pEGFP-C1	Clontech	Bcl-2	Express GFP-Bcl2
E.coli	p3X Flag-NOXA	p3X-Flag-Myc-CMV	Sigma	NOXA	Express Flag-NOXA
E.coli	EGR-1 in 3X-Flag	p3X-Flag-Myc-CMV	Sigma	EGR-I	Express Flag-EGR1
E.coli	SP1 in pCDNA3	pCDNA-3	Invitrogen	SP-I	Express SP1
E.coli	CHOP-in GFP	pEGFP-C1	Clontech	CHOP	Express GFP-CHOP
E.coli	ATF-4 in GFP	pEGFP-C1	Clontech	ATF-4	Express -GFP-ATF4
E.coli	GSK3-beta in pCDNA3	pCDNA-3	Invitrogen	GSK-3-Beta	Express GSK3-Beta
E.coli	FOXO3-CA in pCDNA3	pCDNA-3	Invitrogen	FOXO-3CA	Express FOXO3-CA
E.coli	pHA-PUMA in pEGFP	pEGFP-C1	Clontech	PUMA	Express GFP-PUMA
E.coli	pHA-PUMA in pECFP	pECFP-C1	Clontech	PUMA	Express CFP-PUMA
E.coli	pHA-PUMA in pEYFP	pEYFP-C1	Clontech	PUMA	Express YFP-PUMA
E.coli	pHA-PUMA in pERFP	pERFP-C1	Clontech	PUMA	Express RFP-PUMA
E.coli	Human EGR1DN in 3X Flag	p3x FLAG-CMV	Sigma	EGR1DN	Express FLAG-EGR1DN
E.coli	EGR-1 ZN Finger in 3X Flag	p3x FLAG-CMV	Sigma	EGR1-ZN Finger	Express FLAG-EGR1-Zn Finger
E.coli	SP1 in 3X Flag	p3x FLAG-CMV	Sigma	SP1	Express FLAG-SP1

Bacteria used	Plasmid:	Plasmid	Source of plasmid	Gene Transfected	Change
E.coli	Bim cDNA in pEGXT 4T	pEGXT 4T	Cregan Lab	Bim-L	Express GST-BimL
E.coli	PUMA cDNA in pEGXT 4T	pEGXT 4T	Cregan Lab	PUMA	Express GST-PUMA
E.coli	Bcl XL in GFP C1	pEGFP-C1	Cregan Lab	Bcl XL	Express GFP-BclXL
E.coli	Bcl XL in pEGXT 2T	pEGXT 2T	Cregan Lab	Bcl XL	Express GST-BclXL
E.coli	Bcl 2 in GFP C3	pEGFP-C3	Clontech	Bcl 2	Express GFP-Bcl2
E.coli	Bax D33A Mutant in GFP C3	pEGFP-C3	Clontech	Bax	Express GFP-Bax
E.coli	pSHUTTLE	pSHUTTLE	Cregan Lab		No Change
E.coli	pSHUTTLE CMV	pSHUTTLE CMV	Cregan Lab		No Change
E.coli	pADTRACK	pADTRACK	Cregan Lab		No Change
E.coli	pADTEACK CMV	pADTEACK CMV	Cregan Lab		No Change

Note: All these plasmids are used to study functioning and cell localization of the genes of interest.
None of these plasmids are used for cell transformations.

invitrogen™

Attachment 4

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.

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

End of Safety Data Sheet

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

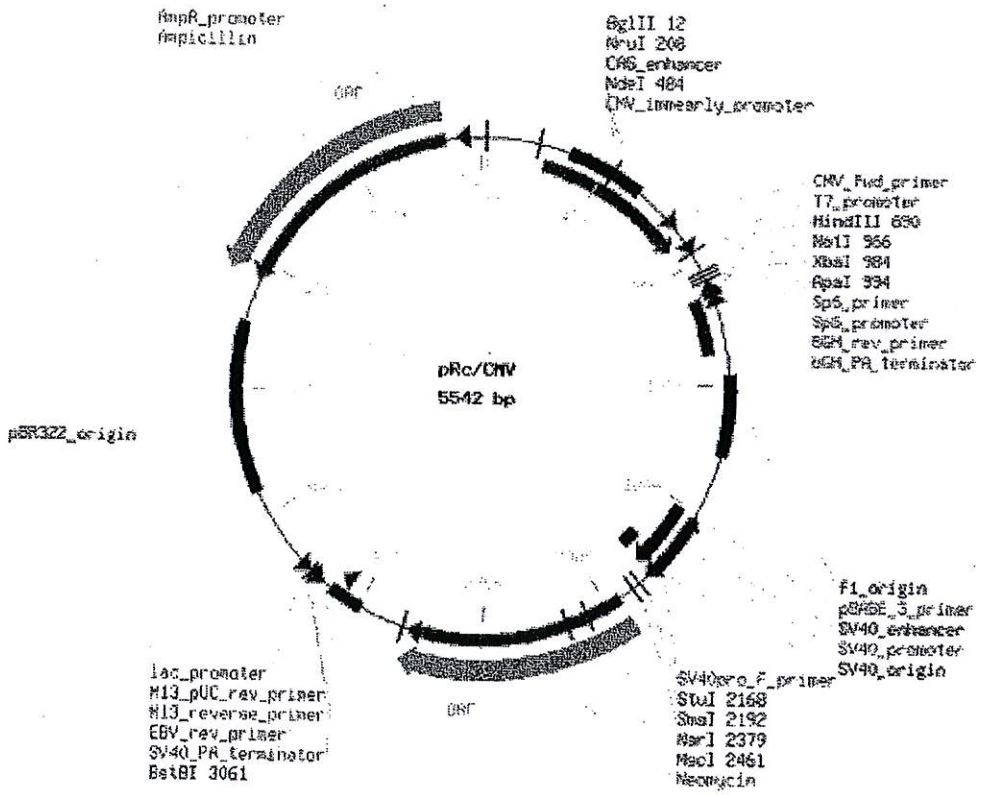
Further information

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 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



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.

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada