THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 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 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. J. G. Pickering
SIGNATURE	
DEPARTMENT	Vascular Biology
ADDRESS	Robarts Research Institute, Room 4245D
PHONE NUMBER	(519)663-5777 x24214
EMERGENCY PHONE NUMBER(S)	
EMAIL	gpickering@robarts.ca

Location of experimental work to be carried out: Building(s) RRI, Med Science Room(s) 4244, 4250, ACVS

*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: CIHR & HSFO
GRANT TITLE(S): Smooth Muscle Cells and Vascular Disease

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Caroline O'Neil	Faran Vafaie
Zengxuan Nong	Paul Comartin
Theodore Small	Alanna Watson
Matthew Frontini	Oula Akawi

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

Research Summary

The purpose of this study is to ascertain molecular details of processes that contribute to the development of diseased arteries and the formation of new blood vessels. Smooth muscle cells, fibroblasts and endothelial cells are prominent constituents of the human atherosclerotic plaque and will be utilized to study their role in vascular disease. These cells are particularly abundant in lesions that rapidly develop after angioplasty-induced vascular injury. Functionally, these cells contribute to atherosclerosis by replicating within the growing lesion, and by synthesizing and secreting extracellular matrix. We will specifically examine molecules, such as Nampt, WTAP and FGF-9, which enable cells in the artery to organize and stabilize the artery wall. These target genes will be inserted into various plasmids and nucleofected into the different cells lines to elucidate their role. Nucleofection protocol will prevent the production of virus. In addition, the mouse and rat animal model can mimic the complex events that take place in individuals with diseased arteries. Use of an animal system enables the retrieval of suitable amounts of tissue, at defined stages of the disease, so that a thorough analysis can be performed. To determine the response of the candidate genes to vascular injury, the carotid artery of rats or the femoral artery of mice, will be injured. Adenovirus will be administered locally to the site of mechanical injury of the artery during surgery and gene expression will be evaluated during various stages of lesion development. The adenovirus will be produced in a level 2 facility at Robarts and the mice will be housed in a level 2 facility at ACVS. The messenger RNA will be assessed by Laser Capture Microdissection and Real Time RT-PCR. Protein elaboration will be assessed immunohistochemically. This proposal aims to elucidate the role of selected genes that are expressed in the vascular lesions which may be critical to lesion development or angiogenesis.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES ⊗ 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, DH5a competent cells	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	2	Invitrogen	⊗ 1 O 2 O 3
AdMax: adenovirus type 5	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	0.1	Microbix Biosystems Inc	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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	⊗ Yes O No	Primary smooth muscle cells derived from arteries	Not applicable
Rodent	⊗ Yes O No	Fibroblasts derived from mouse embryos	2006-064-08
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ 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:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⊗ Yes O No	HEK 293, HAEC, Hela, HT-1080, Fibroblasts (transformed)	ATCC and Lonza
Rodent	⊗ Yes O No	Renca, 3T3-L1, 3T3-Swiss albino, C3H/10T 1/2	ATCC
Non-human primate	⊗ Yes O No	Cos-7	ATCC
Other (specify)	O 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 O 1 ⊗ 2 O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Dr. Michael Chu LHSC	O Yes O No ⊗ Unknown		O 1 ⊗ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)	Dr. Michael Chu LHSC	O Yes O No ⊗ Unknown		O 1 ⊗ 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ⊗ 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
E.coli DH5alpha competent cells	pEGFP-N3, pIRES2-EGFP, pLNCX2, pQCXIN, pQCXIP	Clontech, addgene	Genes involved in vascular disease such as Nampt, WTAP & FGF-9	Genes play a role in the development of vascular lesions

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Admax (adenovirus type 5)	Adeno E1A, Admax vectors (pD311, pDC411, pDC511)	Microbix Biosystems	Genes involved in vascular disease such as Nampt, WTAP & FGF-9	Genes play a role in the development of vascular lesions

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ⊗ YES O NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used mouse, rat

6.3 AUS protocol # 2006-064-08

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

6.5 Will the agent(s) be shed by the animal: ⊗ YES O NO, please justify:

_____

_____

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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*? _____

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

*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 life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

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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
If NO, please forward the permit to he Biosafety Officer when available.

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 12.0 ⊗ 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 _____

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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 ⊗ 2 O 3

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

⊗ YES, permit # if on-campus BIO-RRI-0028
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 J. S. Pilon Date: Feb 18, 2010

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

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

Health and safety protocol: report to first-aid person and follow-up with university occupational health and safety

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: Randall Norkosky
Date: Feb. 24, 2010

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***

invitrogen

Material Safety Data Sheet

Revision Date: 09-Nov-2006

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

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
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

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	5-10

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

3. HAZARDS IDENTIFICATION

Emergency Overview

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
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

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	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

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
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

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

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Glycerol	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65
This product contains the following Proposition 65 chemicals:

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

Public Health Agency of Canada | Agence de la santé publique du Canada

Canada

Adenovirus types 1, 2, 3, 4, 5 and 7 - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Adenovirus types 1, 2, 3, 4, 5 and 7***

SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

CHARACTERISTICS: *Adenoviridae*; non-enveloped, icosahedral virions, 70-90 nm diameter, doubled-stranded, linear DNA genome.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is associated with adenovirus infection

EPIDEMIOLOGY: Worldwide; seasonal in temperate regions, with highest incidences in the fall, winter and early spring; in tropical areas, infections are common in the wet and colder weather; annual incidence is particularly high in children; adenovirus types 4 and 7 are common among military recruits (ARD)

HOST RANGE: Humans

INFECTIOUS DOSE: >150 plaque forming units when given intranasally

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal-oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available; cidofovir has shown promise in the treatment of adenoviral ocular infections.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2%

glutaraldehyde, 0.25% sodium dodecyl sulfate

PHYSICAL INACTIVATION: Sensitive to heat>56°C; unusually stable to chemical or physical agents and adverse pH conditions

SURVIVAL OUTSIDE HOST: Resistance to chemical and physical agents allows for prolonged survival outside of the body. Adenovirus type 3 survived up to 10 days on paper under ambient conditions; adenovirus type 2 survived from 3-8 weeks on environmental surfaces at room temperature

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

PROPHYLAXIS: None available

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Ten cases documented up to 1988

SOURCES/SPECIMENS: Respiratory secretions

PRIMARY HAZARDS: Ingestion; droplet exposure of the mucous membrane

SPECIAL HAZARDS: Contact with feces from infected animals

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

PROTECTIVE CLOTHING: Laboratory coat; gloves when skin contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

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

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-01-23

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

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I

Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II

Physical data

Pink or red aqueous liquid

SECTION III

Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV

Fire and explosion

Not applicable

SECTION V
Reactivity data

Stable. Hazardous polymerization will not occur.

SECTION VI
Method of disposal

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII
Special protection information

For Biosafety Level 1 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII
Special precautions or comments

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

February 2002

Cell Biology

ATCC® Number:	**CRL-1573™** Order this Item	Price:	**$256.00**
Designations:	293 [HEK-293]		
Depositors:	FL Graham		
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial 		
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.		
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]		
Receptors:	vitronectin, expressed		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19		

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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
Comments:	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. 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.

Preservation:

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

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 30-2003
derivative: ATCC CRL-10852
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

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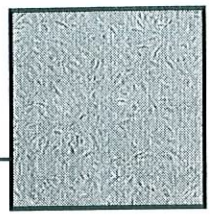
Clonetics® Aortic Endothelial Cell Systems



HAEC

Introduction

Clonetics® Aortic Endothelial Cell Systems contain Normal Human Aortic Endothelial Cells (HAEC) and optimized media for their growth. Each System can quickly generate HAEC cultures for experimental applications in cardiovascular pharmaceutical development and vascular pathology, including atherosclerosis. Clonetics® Aortic Endothelial Cell Systems are convenient and easy to use, allowing the researcher to focus on results. Cryopreserved HAEC are shipped in third passage. Proliferating HAEC are shipped in fourth passage.

Clonetics® Cells, Medium and Reagents are quality tested together and guaranteed to give optimum performance as a complete Cell System.

Cell System Components

- One Aortic Endothelial Cell Product (Cryopreserved or Proliferating)
- Clonetics® EGM®-2 BulletKit® (CC-3162) contains one 500 ml bottle of Endothelial Cell Basal Medium-2 and the following growth supplements: Hydrocortisone, 0.2 ml; hFGF-B, 2 ml; VEGF, 0.5 ml; R^3-IGF-1, 0.5 ml; Ascorbic Acid, 0.5 ml; Heparin, 0.5 ml; FBS, 10 ml; hEGF, 0.5 ml; GA-1000, 0.5 ml.
- One ReagentPack™ (CC-5034) Containing:

Trypsin/EDTA	100 ml
Trypsin Neutralizing Solution	100 ml
HEPES Buffered Saline Solution	100 ml

Characterization of Cells

Routine characterization of HAEC includes immunofluorescent staining. Cells stain positive for acetylated LDL and von Willebrand (Factor VIII) antigen. Cells stain negative for smooth muscle α-actin.

Performance

Recommended seeding density for subculture	2,500 - 5,000 cells/cm^2
Typical time from subculture to confluent monolayer	5 - 9 days
Additional population doublings guaranteed using Clonetics® System	15

Quality Control

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity is measured after recovery from cryopreservation. Clonetics® Media are formulated for optimal growth of specific types of normal human cells. Each lot of medium is tested for the support of cell viability and proliferative capacity. Certificates of Analysis (CA) for each cell strain are shipped with each order. CA for all other products are available upon request.

Ordering Information

Cryopreserved Cells

CC-2535	HAEC	≥ 500,000 cells

Proliferating Cells – Flasks and Multiwell Plates

CC-2635	T-25 Flask
CC-0222	T-75 Flask
CC-0132	96-well Plate

Other proliferating formats are available. Contact Technical Service or refer to the Lonza website for details.

CC-3162	EGM®-2 BulletKit®, EBM®-2 plus SingleQuots® of Growth Supplements	500 ml
CC-3156	EBM®-2, Endothelial Basal Medium-2	500 ml
CC-4176	EGM®-2 SingleQuots®, Formulates EBM®-2 to EGM®-2	
CC-5034	ReagentPack™	
	Trypsin Neutralizing Solution	100 ml
	Trypsin/EDTA Solution	100 ml
	HEPES Buffered Saline Solution	100 ml

Lonza

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza guarantees the performance of its cells only if Clonetics® Media and Reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete Cell System. Cryopreserved HAEC are assured to be viable and functional when thawed and maintained properly.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Technical Services.

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
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Cell Biology

ATCC® Number: CCL-2™ Order this Item
Price: $256.00

Designations: HeLa

Depositors: WF Scherer

Biosafety Level: 2 [CELLS CONTAIN PAPOVAVIRUS]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

PHOTO

Source:
Organ: cervix
Disease: adenocarcinoma
Cell Type: epithelial

Cellular Products:
keratin
Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]

Permits/Forms:
In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Applications:
transfection host ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
screening for Escherichia coli strains with invasive potential [21447] [21491]

Virus Susceptibility:
Human adenovirus 3
Encephalomyocarditis virus
Human poliovirus 1
Human poliovirus 2
Human poliovirus 3

Reverse Transcript: negative

DNA Profile (STR):
Amelogenin: X
CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10
D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

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Cytogenetic Analysis: Modal number = 82; range = 70 to 164.
There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of MI, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.

Isoenzymes: G6PD, A

Age: 31 years adult

Gender: female

Ethnicity: Black

HeLa Markers: Y

Comments: The cells are positive for keratin by immunoperoxidase staining.
HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences.
P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.

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

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 which 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 1:2 to 1:6 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 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CCL-2.1
derivative:ATCC CCL-2.2
derivative:ATCC CCL-2.3

References:

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Cell Biology

ATCC® Number:	**CCL-121™** Order this Item	**Price:**	**$272.00**
Designations:	HT-1080		**Related Links**
Biosafety Level:	1		NCBI Entrez Search
Shipped:	frozen		Make a Deposit
Medium & Serum:	See Propagation		Frequently Asked Questions
Growth Properties:	adherent		Material Transfer Agreement
Organism:	*Homo sapiens* (human)		Technical Support
Morphology:	epithelial		Related Cell Culture Products
Source:	**Tissue:** connective tissue **Disease:** fibrosarcoma		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Isolation:	**Isolation date:** July, 1972		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Virus Susceptibility:	Human poliovirus 1 RD-114 Feline Feline leukemia virus Vesicular stomatitis virus		
Tumorigenic:	Yes		
Reverse Transcript:	negative		
Oncogene:	ras +		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 12,14 D16S539: 9,12 D5S818: 11,13 D7S820: 9,10 THO1: 6 TPOX: 8 vWA: 14,19		
Cytogenetic Analysis:	modal number = 46; range = 44 to 48. Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.		
Isoenzymes:	G6PD, B		
Age:	35 years		
Gender:	male		
Ethnicity:	Caucasian		

Comments: The cells contain an activated N-ras oncogene.

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%.
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: 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: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020

References:

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Return to Top

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Cell Biology

ATCC® Number:	**CRL-2947™** Order this Item	**Price:**	**$355.00**
Designations:	Renca		
Depositors:	R.Wiltrout		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Mus musculus* (mouse)		
Morphology:	epithelial-like 		
Source:	**Organ:** kidney **Disease:** renal adenocarcinoma **Cell Type:** epithelial **Strain:** Balb/cCr		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Isolation:	Isolation date: 1969		
Tumorigenic:	YES		
Age:	6 weeks		
Gender:	male		
Comments:	The Renca cell line was derived from a tumor that arose spontaneously as a renal cortical adenocarcinoma in Balb/cCr mice. The pattern of growth of this tumor accurately mimics that of human adult renal cell carcinoma, particularly with regard to spontaneous metastasis to lung and liver. [PubMed: 4703766, 4057425]The cells do not express transforming growth factor-beta type II receptor (TbetaR-II). [PubMed: 10414746]		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: • 10% fetal bovine serum (final conc.) • non-essential amino acids (NEAA) (0.1mM extra) • additional sodium pyruvate (1mM extra) • additional L-glutamine (2mM extra) **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5%		

Related Links

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Subculturing:

Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which 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. An inoculum of 2 X 10(4) to 4 X 10(4) viable cells/sq. cm is recommended.
6. Incubate cultures at 37C. We recommend that you subculture when the culture reaches a cell concentration between 8 X 10(4) and 1.5 X 10(5) cells/sq. cm.
   Subcultivation ratio: A subcultivation ratio of 1:4 to 1:10 is recommended.
   Medium renewal: Every 2 to 3 days

Preservation:

Freeze medium: RPMI-1640 Medium, 77.5%; FBS, 15% FBS; DMSO, 7.5%
Storage temperature liquid nitrogen vapor phase

Doubling Time:

approximately 24 hours

Related Products:

Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2001
Recommended serum: ATCC 30-30-2020
0.25% (w/v) Trypsin - 0.53mM EDTA in Hank's BSS (w/o Ca++, Mg ++): ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X
L-Glutamine solution, 200mM: ATCC 30-2214
Erythrosin B vital stain solution: ATCC 30-2404

References:

16172681: Murphy GP, Hrushesky WJ. A murine renal cell carcinoma. J. Natl. Cancer Inst. 50(4):1013-25, 1973. PubMed: 4703760
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16172683: Engel J, et al. Transforming growth factor-beta type II receptor confers tumor suppressor activity in murine renal carcinoma (renca) cells . Urology. 54(1):164-70, 1999. PubMed: 10414746

Return to Top

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Cell Biology

ATCC® Number: CL-173™ Order this Item
Price: $256.00

Designations: 3T3-L1

Depositors: Massachusetts Institute of Technology

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: fibroblast

PHOTO

Source: **Organ:** embryo
Cell Type: fibroblast

Cellular Products: triglycerides [3491]

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

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

Receptors: insulin, expressed

Reverse Transcript: negative

Age: embryo

Comments: L1 is a continuous substrain of 3T3 (Swiss albino) developed through clonal isolation. The cells undergo a pre-adipose to adipose like conversion as they progress from a rapidly dividing to a confluent and contact inhibited state. A high serum content in the medium enhances fat accumulation [PubMed ID: 4426090].
Tested and found negative for ectromelia virus (mousepox).
This line is also designated as ATCC CCL-92.1.ATCC CL-173 was deposited in 1974 without passage number information from the depositor. At the time of submission, ATCC prepared approximately 30 vials of seed stock at about 4 passages beyond the original depositor material (passage number: unknown +4).

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: bovine calf serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum.

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Subculturing:

Protocol: Never allow culture to become completely confluent.

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 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.
   The recommended inoculum is 2 to 3 X 10(3) cells/sq. cm. Subculture before cultures become 70 to 80% confluent or when cells reach 5 to 6 X10(4) viable cells/sq. cm.
6. Incubate cultures at 37C.

Preservation:

Interval: Every three days
Medium Renewal: 2 to 3 times per week
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 14 hrs

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
formerly distributed as:ATCC CCL-92.1
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
Recommended serum: ATCC 30-2030

References:

886: Green H, Meuth M. An established pre-adipose cell line and its differentiation in culture. Cell 3: 127-133, 1974. PubMed: 4426090
3491: Green H. Triglyceride-accumulating clonal cell line. US Patent 4,003,789 dated Jan 18 1977
32373: Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260
32455: Scherer PE, et al. Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. Proc. Natl. Acad. Sci. USA 93: 131-135, 1996. PubMed: 8552590
32787: Kallen CB, Lazar MA. Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. USA 93: 5793-5796, 1996. PubMed: 8650171

Return to Top

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Cell Biology

ATCC® Number:	**CCL-92™** Order this Item
Designations:	3T3-Swiss albino
Depositors:	H Green
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Cell Type:** fibroblast
Cellular Products:	Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1962
Virus Susceptibility:	polyomavirus; SV40
Reverse Transcript:	negative
Cytogenetic Analysis:	This is a hypertriploid mouse cell line. The modal chromosome number was 68 occurring in 30% of cells. The rate of cells with higher ploidies was 2.4%.
Age:	embryo
Comments:	The 3T3 cell line was established by G. Todaro and H. Green in 1962 from disaggregated Swiss mouse embryos. [5732] The cells are contact inhibited. A confluent monolayer yields 40000 cells/sq cm. Tested and found negative for ectromelia virus (mousepox). The cells should be grown in plastic flasks, they do not grow well on some types of glass surfaces. A saturation density of approximately 50000 cells per sq cm can be reached.
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: bovine calf serum to a final concentration of 10%. **Temperature:** 37.0°C

Price: **$256.00**

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Subculturing:	**Protocol:** Never allow culture to become completely confluent. Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. For plates (50mm) use an inoculum of 3 X 10 exp5 cells per plate and subculture every 3 days. For 75 sq cm flasks use 4 X 10 exp5 cells per flask and subculture every 3 days. **Medium Renewal:** Twice per week
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Doubling Time:	18 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 irradiated to be used as feeder cells:ATCC 48-X
References:	5732: Todaro GJ, Green H. Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. J. Cell Biol. 17: 299-313, 1963. PubMed: 13985244 21632: Bennicelli JL, et al. Mechanism for transcriptional gain of function resulting from chromosomal translocation in alveolar rhabdomyosarcoma. Proc. Natl. Acad. Sci. USA 93: 5455-5459, 1996. PubMed: 8643596 26261: Vogt M, Dulbecco R. Studies on cells rendered neoplastic by polyoma virus: the problem of the presence of virus-related materials. Virology 16: 41-51, 1962. PubMed: 13975482 26262: Todaro GJ, et al. Antigenic and cultural properties of cells doubly transformed by polyoma virus and SV40. Virology 27: 179-185, 1965. PubMed: 4284655 26263: Todaro GJ, et al. Transformation of properties of an established cell line by SV40 and polyoma virus. Proc. Natl. Acad. Sci. USA 51: 66-73, 1964. PubMed: 14104605 26623: Fang X, et al. Lysophosphatidylcholine stimulates activator protein 1 and the c-Jun N-terminal kinase activity. J. Biol. Chem. 272: 13683-13689, 1997. PubMed: 9153219 32307: Chen ST, et al. Generation of packaging cell lines for pseudotyped retroviral vectors of the G protein of vesicular stomatitiis virus by using a modified tetracycline inducible system. Proc. Natl. Acad. Sci. USA 93: 10057-10062, 1996. PubMed: 8816750 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 33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

Return to Top

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Cell Biology

ATCC® Number:	**CCL-226™** Order this Item	**Price:**	**$269.00**
Designations:	C3H/10T1/2, Clone 8		**Related Links**
Depositors:	C Heidelberger		NCBI Entrez Search
Biosafety Level:	1		Cell Micrograph
Shipped:	frozen		Make a Deposit
Medium & Serum:	See Propagation		Frequently Asked Questions
Growth Properties:	adherent		Material Transfer Agreement
Organism:	*Mus musculus* (mouse)		Technical Support
Morphology:	fibroblast		Related Cell Culture Products
	PHOTO		
Source:	**Strain:** C3H **Organ:** embryo		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Applications:	transfection host (Roche FuGENE® Transfection Reagents)		
Tumorigenic:	No		
Reverse Transcript:	negative		
Antigen Expression:	H-2k		
Cytogenetic Analysis:	Mouse karyotype with a modal number of 80 chromosomes.		
Age:	embryo		

Comments:	C3H/10T1/2, Clone 8 was isolated by C. Reznikoff, D. Brankow and C. Heidelberger in 1972 from a line of C3H mouse embryo cells. [23019] The cells are very sensitive to post confluence inhibition of cell division, do not produce tumors in syngeneic mice, have no background of spontaneous transformation, nor do they contain overt endogenous transforming murine leukemia or sarcoma viruses. [22697] The cells are contact sensitive. There is no detectable background spontaneous transformation. They are highly susceptible to transformation by chemical agents. [1208] Tested and found negative for ectromelia virus (mousepox). NOTE: THE INOCULATION DENSITY, FEEDING AND HARVESTING SCHEDULES MUST BE FOLLOWED RIGIDLY IF THE LINE IS TO RETAIN ITS ESSENTIAL CHARACTERISTICS. THE BATCH OF SERUM USED FOR GROWTH AND FOR TRANSFORMATION ASSAYS MAY AFFECT BOTH THE MORPHOLOGY OF THIS LINE AND THE RESULTS OBTAINED. Monolayers established and maintained for the standard transformation assay should be free of all foci after 6 weeks. [1208] The donor recommends that the line be used between the 5th and 15th passages only.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is Eagle's Basal medium with 2 mM L-glutamine , 1.5 g/L sodium bicarbonate and Earle's BSS. To make the complete growth medium, add the following components to the base medium: heat-inactivated fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. SUBCULTURE MUST BE DONE BEFORE THE CULTURE REACHES CONFLUENCE. **Subcultivation Ratio:** Seed new flasks at 2000 viable cells/sq cm. **Medium Renewal:** Once between subcultures if necessary
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid notrogen vapor temperature
References:	1208: Reznikoff CA, et al. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. Cancer Res. 33: 3239-3249, 1973. PubMed: 4795600 1209: Terzaghi M, Little JB. Repair of potentially lethal radiation damage in mammalian cells is associated with enhancement of malignant transformation. Nature 253: 548-549, 1975. PubMed: 1167940 1210: Mondal S, Heidelberger C. Transformation of C3H/10T1/2 CL8 mouse embryo fibroblasts by ultraviolet irradiation and a phorbol ester. Nature 260: 710-711, 1976. PubMed: 1264212 22440: Smith GJ, et al. Clonal analysis of the expression of multiple transformation phenotypes and tumorigenicity by morphologically transformed 10T1/2 cells. Cancer Res. 53: 500-508, 1993. PubMed: 8425185 22697: Rapp UR, et al. Endogenous oncomaviruses in chemically induced transformation. I. Transformation independent of virus production. Virology 65: 392-409, 1975. PubMed: 165619 23019: Reznikoff CA, et al. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer Res. 33: 3231-3238, 1973. PubMed: 4352355 33039: Jain MK, et al. Molecular cloning and characterization of SmLIM, a developmentally regulated LIM protein preferentially expressed in aortic smooth muscle cells. J. Biol. Chem. 271: 10194-10199, 1996. PubMed: 8626582

Return to Top

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Cell Biology

ATCC® Number: CRL-1651™ Order this Item
Price: $269.00

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

Organism: *Cercopithecus aethiops*

Morphology: fibroblast

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.

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

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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: 8570613
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
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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
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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. PubMed: 8702465
33011: Ozcelebi F, et al. Phosphorylation of cholecystokinin receptors expressed on chinese hamster ovary cells. J. Biol. Chem. 271: 3750-3755, 1996. PubMed: 8631990
33013: Gibson S, et al. Functional LCK is required for optimal CD28-mediated activation of the TEC family tyrosine kinase EMT/ITK. J. Biol. Chem. 271: 7079-7083, 1996. PubMed: 8636141
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33137: Arai H, Charo IF. Differential regulation of G-protein-mediated signaling by chemokine receptors. J. Biol. Chem. 271: 21814-21819, 1996. PubMed: 8702980
33163: Hsieh CM, et al. APEG-1, a novel gene preferentially expressed in aortic smooth muscle cells, is down-regulated by vascular injury. J. Biol. Chem. 271: 17354-17359, 1996. PubMed: 8663419
33175: Holtmann MH, et al. Multiple extracellular loop domains contribute critical determinants for agonist binding and activation of the secretin receptor. J. Biol. Chem. 271: 14944-14949, 1996. PubMed: 8663161

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Back to my Search

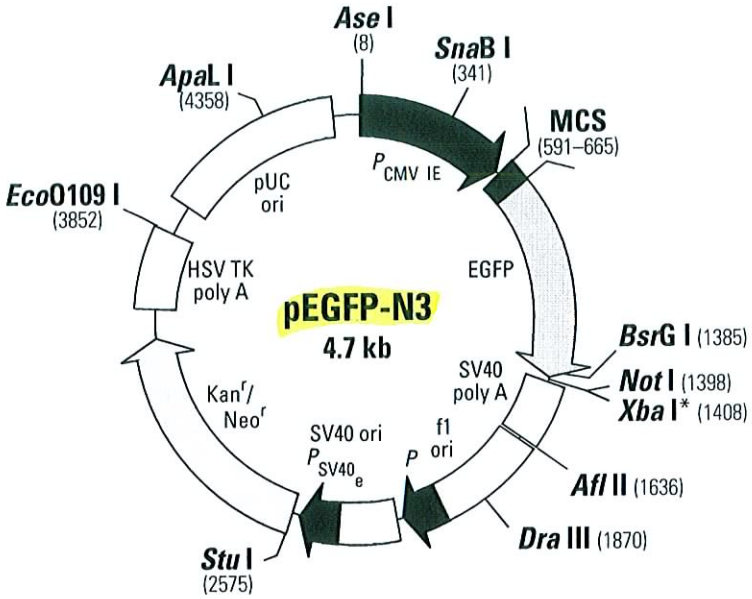
Vector Information

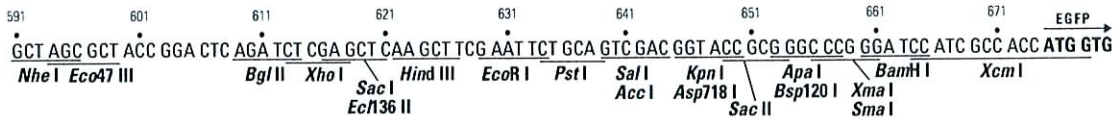
pEGFP-N3 Vector Information

PT3054-5

GenBank Accession #: U57609

Catalog #6080-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N3 (Unique restriction sites are in bold). The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-N3 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-N3 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-N3 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (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-N3 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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United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR29967; published 03 October 2002)

Use:
Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N3 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N3 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of Features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465
  TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MCS: 591–665
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 668–678
  Start codon (ATG): 675–677; Stop codon: 1392–1394
  Insertion of Val at position 2: 678–680
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 867–872
  His-231 to Leu mutation (A→T): 1369
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1548–1553 & 1577–1582; mRNA 3' ends: 1586 & 1598
- f1 single-strand DNA origin: 1645–2100 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2162–2167; –10 region: 2185–2190
  Transcription start point: 2197
- SV40 origin of replication: 2441–2576
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2274–2345 & 2346–2417
  21-bp repeats: 2421–2441, 2442–2462 & 2464–2484
  Early promoter element: 2497–2503
  Major transcription start points: 2493, 2531, 2537 & 2542
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2625–2627; stop codon: 3417–3419
  G→A mutation to remove *Pst* I site: 2807
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3153
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3655–3660 & 3668–3673
- pUC plasmid replication origin: 4004–4647

Primer Locations:
- EGFP-N Sequencing Primer (#6479-1): 741–720
- EGFP-C Sequencing Primer (#6478-1): 1328–1349

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.

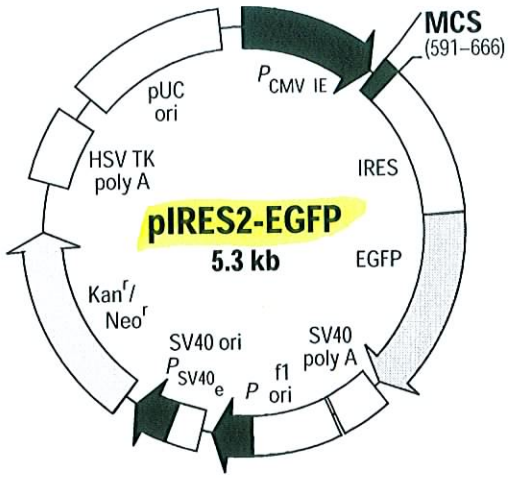
Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

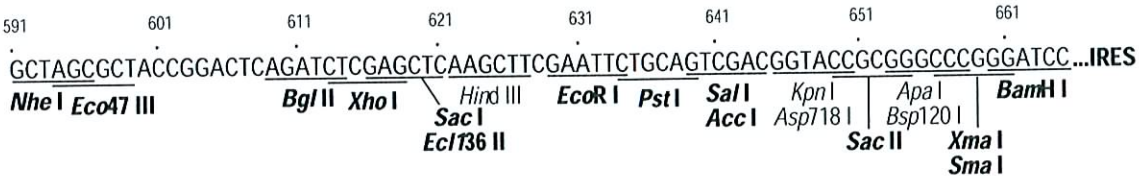
Vector Information

pIRES2-EGFP Vector Information

PT3267-5

Catalog #6029-1





Restriction Map and Multiple Cloning Site (MCS) of pIRES2-EGFP Vector. Unique restriction sites are in bold. Note that the *Eco*47 III site has not been confirmed in the final construct.

Description:

pIRES2-EGFP contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and the enhanced green fluorescent protein (EGFP) coding region. This permits both the gene of interest (cloned into the MCS) and the EGFP gene to be translated from a single bicistronic mRNA. pIRES2-EGFP is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing EGFP and the protein of interest. This vector can also be used to express EGFP alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

EGFP is a red-shifted variant of wild-type GFP (3–5) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) EGFP encodes the GFPmut1 variant (6) 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 (7). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (8) to further increase the translation efficiency in eukaryotic cells. The MCS in pIRES2-EGFP is between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the bicistronic mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pIRES2-EGFP backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. pIRES2-EGFP replaces (but is not derived from) the pIRES-EGFP Vector previously sold by BD Biosciences Clontech. pIRES2-EGFP is functionally similarly to pIRES-EGFP; however, pIRES2-EGFP gives brighter EGFP fluorescence than the older vector. Note that the *Xba* I site at position

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
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1987 is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Use:
Genes inserted into the MCS should include the initiating ATG codon. pIRES2-EGFP and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

Location of features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MCS: 591–665
- IRES sequence: 666–1250
- Enhanced green fluorescent protein (EGFP) gene
  Kozak consensus translation initiation site: 1247–1257
  Start codon (ATG): 1254–1256; Stop codon: 1971–1973
  Insertion of Val at position 2: 1257–1259
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 1446–1451
  His-231 to Leu mutation (A→T): 1948
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2127–2132 & 2156–2161; mRNA 3' ends: 2165 & 2177
- f1 single-strand DNA origin: 2224–2679 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2741–2746; –10 region: 2764–2769
  Transcription start point: 2776
- SV40 origin of replication: 3020–3155
- SV40 early promoter/enhancer
  72-bp tandem repeats: 2853–2996; 21-bp repeats (3): 3000–3063
  Early promoter element: 3076–3082
- Kanamycin/neomycin resistance gene: 3204–3998
  G→A mutation to remove *Pst* I site: 3386; C→A (Arg to Ser) mutation to remove *Bss*H II site: 3732
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4234–4252
- pUC plasmid replication origin: 4583–5226

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 JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:
1. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**:477–483.
2. Jang, S. K., *et al.* (1990) *J. Virol.* **62**:2636–2643.
3. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
4. Yang, T. T., *et al.* (1996) *Nucleic Acids Res.* **24**:4592–4593.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Jackson, R. J., *et al.* (1990) *Trends Biochem.* **15**:477–483.
7. Jang, S. K., *et al.* (1988) *J. Virol.* **62**:2636–2643.
8. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947.
9. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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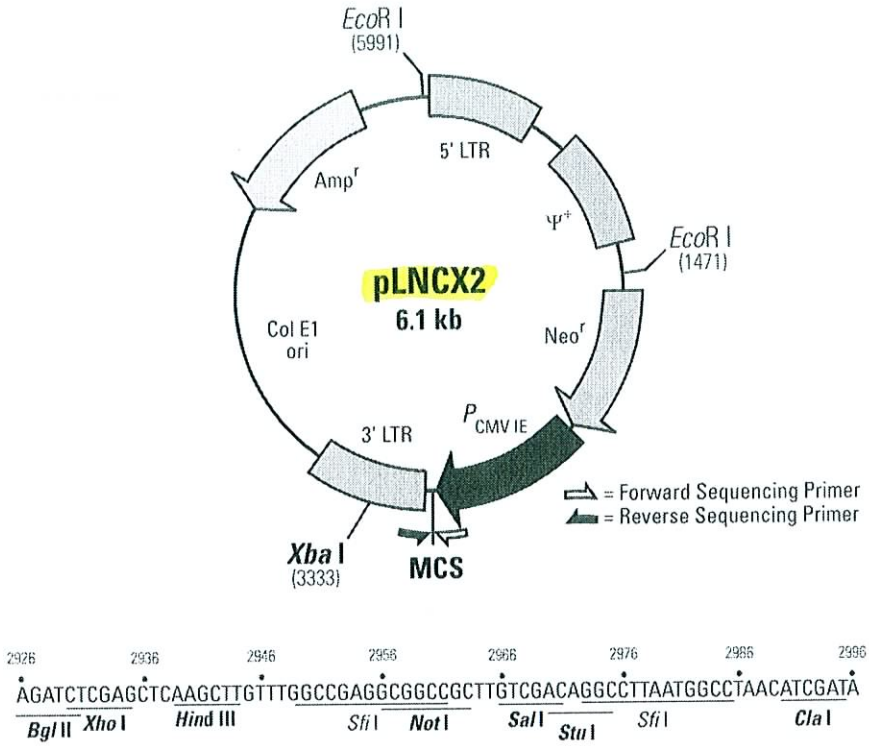
Vector Information

pLNCX2 Vector Information

PT3297-5

GenBank Accession #: Submission in progress.

Cat. No. 631503
631508
631511



Restriction Map and Multiple Cloning Site (MCS) of pLNCX2. Unique restriction sites are in bold.

Description

pLNCX2 contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression (1–3). Upon transfection into a packaging cell line, pLNCX2 can transiently express, or integrate and stably express, a transcript containing Ψ+ (the extended viral packaging signal) a selectable marker, and the gene of interest. The 5' viral LTR in this vector contains viral promoter/enhancer sequences that control expression of the neomycin resistance (Neo^r) gene for antibiotic selection in eukaryotic cells. A gene of interest can be cloned into the multiple cloning site immediately downstream of the human cytomegalovirus (CMV) immediate early promoter (P_{CMV}). pLNCX2 also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pLNCX2 can be transfected into a packaging cell line such as the RetroPack™ PT67 Cell Line (Cat. No. 631510). Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLNCX2 does not contain the structural genes (*gag, pol,* and *env)* necessary for particle formation and replication; these genes are stably integrated into PT67 (4–7). Subsequent introduction of pLNCX2, containing Ψ+, transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. These retroviral particles can infect target cells and transmit the gene of interest (which is cloned between the viral LTR sequences), but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

(PR792366; published 10 September 2007)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
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Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG–>TAG): 1049–1051
- Neomycin resistance gene (Neo^r):
  Start codon: 1512–1514; stop codon: 2304–2306
- Immediate early CMV promoter (P_{CMV}): 2374–2906
- Multiple Cloning Site (MCS): 2926–2996
- 3' MoMuLV LTR: 3035–3628
- Col E1 origin of replication:
  Site of replication initiation: 4164
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5784–5782; stop codon: 4926–4924

Sequencing primer locations

- pLNCX Seq/PCR Primers:
  5' primer (2882-2906): 5'-AGCTGGTTTAGTGAACCGTCAGATC-3'
  3' primer (3057-3032): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
4. Mann, R., *et al.* (1983) *Cell* **33**:153–159.
5. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**:2895–2902.
6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
7. Miller, A. D. & Chen, F. (1996) *J. Virol.* **70**:5564–5571.

Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

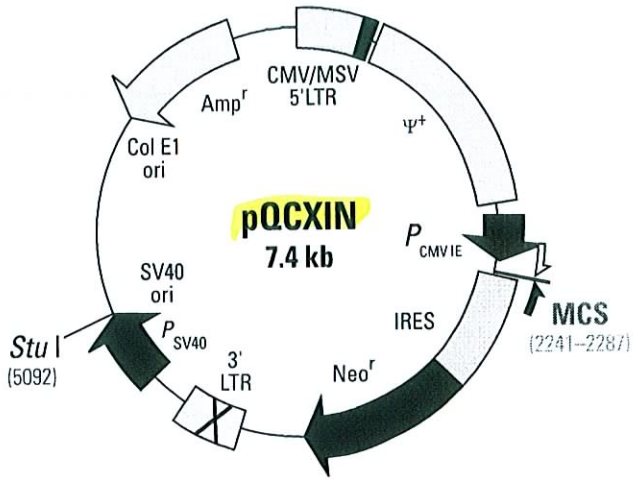
The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

Vector Information

pQCXIN Retroviral Vector Information

PT3667-5

Cat. No. 631514



= Forward Sequencing Primer
= Reverse Sequencing Primer

2240 2250 2260 2270 2280

CGCGGCCGCACCGGTAGGCCTCGTACGCTTAATTAACGGATCCGGAATTCC

Not I *Age* I *Bsi* WI *Pac* I *Bam*H I *Eco*R I

Restriction Map and Multiple Cloning Site (MCS) of pQCXIN Vector. Unique restriction sites are in bold.

Description

pQCXIN Retroviral Vector is a bicistronic expression vector designed to express a target gene along with the neomycin selection marker (1). Upon transfection into a packaging cell line, this vector can transiently express, or integrate and stably express a viral genomic transcript containing the CMV immediate early promoter, gene of interest, IRES and the neomycin resistance gene (Neo^r). The gene of interest and the neomycin resistance gene are co-translated, via the internal ribosome entry site (IRES), from a bicistronic message in mammalian cells (2, 3).

This vector incorporates unique features including: optimization to remove promoter interference and self-inactivation. The hybrid 5' LTR consists of the cytomegalovirus (CMV) type I enhancer and the mouse sarcoma virus (MSV) promoter. This construct drives high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (4, 5, 6, 7) in these cells. The self-inactivating feature of the vector is provided by a deletion in the 3' LTR enhancer region (U3). During reverse transcription of the retroviral RNA, the inactivated 3' LTR is copied and replaces the 5' LTR, resulting in inactivation of the 5' LTR CMV enhancer sequences. This may reduce the phenomenon known as promoter interference (8) and allow more efficient expression.

Also included in the viral genomic transcript are the necessary viral RNA processing elements including the LTRs, packaging signal (Psi$^+$), and tRNA primer binding site. pQCXIN also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria.

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United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
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Use

pQCXIN is designed to deliver and express a gene along with the neomycin resistance marker from a bicistronic message. The design is optimized to produce high titers via the $P_{CMV\ IE}$ in the packaging cell line. The bicistronic transcript makes it unnecessary to screen the transformants since the neomycin resistance is expressed in concert with the DNA inserted into the multiple cloning site.

Once transfected into the packaging cell line (such as the RetroPack™ PT67 Cell Line (Cat. No.631510) AmphoPack293, EcoPack2-293, or Pantropic System), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQCXIN lacks structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated as part of the packaging cell genome. Once a high titer clone is selected, these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate within these cells due to the absence of viral structural genes. The separate introductioin and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recomination events during cell proliferation.

Location of Features

- 5' LTR (CMV/MSV): 1–728
  Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter:1–511
  R region: 584–654
  U5 region: 655–728
- Ψ^+ (extended packaging signal): 758–1567
- Cytomegalovirus (CMV) immediate early promoter ($P_{CMV\ IE}$): 1601–2132
- Multiple Cloning Site (MCS): 2238–2287
- Internal ribosome entry site (IRES): 2289–2862
- Neomycin resistance gene (Neo^r): 2876–3670
- 3' MoMuLV LTR (deletion in U3): 4087–4512
  Poly A signal: 4415–4420
  cleavage site: 4435–4436
- SV40 promoter: 4792–5059
- SV40 ori: 5013–5078
  Site of replication initiation
- Col E1 ori (Site of replication initiation): 5399
- Ampicillin resistance gene (β-lactamase): 7019–6159
  Start codon (ATG): 7019–7017 stop codon (TAA): 6161–6159

Sequencing Primer Locations

- pQC Seq/PCR Primers:
  5' primer (2141–2164): 5'-ACGCCATCCACGCTGTTTTGACCT-3'
  3' primer (2311–2334): 5'-AAGCGGCTTCGGCCAGTAACGTTA-3'

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

References

1. Julius, M. A., Yan, Q., Zheng, Z., & Kitajewski, J. (2000) *BioTechniques* **28**(4):702–707.
2. Adam, M. A., Ramesh, N., Miller, A. D. & Osborne, W. R. (1991) *J. Virol.* **65**:4985–4990.
3. Ghattas, I. R., Sanes, J. R. & Majors, J. E. (1991) *Mol. Cell Biol.* **11**:5848–5859.
4. Kinsella, T. M. & Nolan G. P. (1996) *Hum. Gene Ther.* **7**:1405–1413.
5. Ory, D. S., Neugeboren, B. A. & Mulligan, R. C. (1996) *Proc. Nat. Acad. Sci. USA* **93**:11400–11406.
6. Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**(18):8392–8396.
7. Yang, S., Delgado, R., King, S. R., Woffendin, C., Barker, C. S., Yang, Z. Y., Xu, L., Nolan, G. P. & Nabel, G. J. (1999) *Hum. Gene Ther.* **10**:123–132.
8. Emerman, M. & Temin, H. M. (1984) *Cell* **39**:449–467.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with sequences obtained by Clontech Laboratories, Inc. This vector has been completely sequenced.

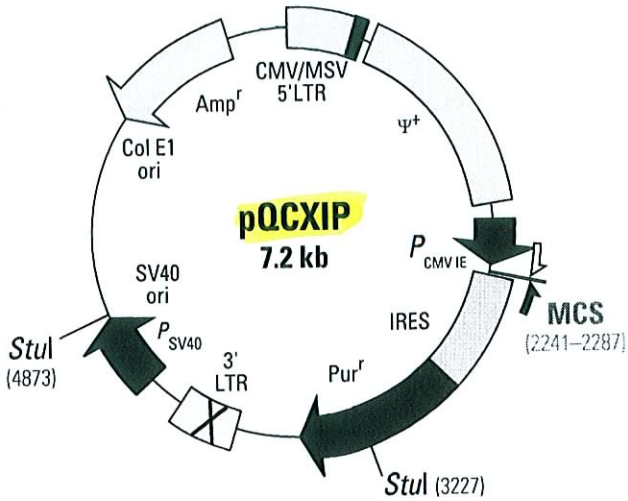
The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

Vector Information

pQCXIP Vector Information

PT3669-5

Cat. No. 631516



= Forward Sequencing Primer
= Reverse Sequencing Primer

2240 2250 2260 2270 2280

CGCGGCCGCACCGGTAGGCCTCGTACGCTTAATTAACGGATCCGGAATTCC

Not I **Age I** **Pac I** **BamH I** **EcoR I**

Restriction Map and Multiple Cloning Site (MCS) of pQCXIP Vector. Unique restriction sites are in bold.

Description

pQCXIP Retroviral Vector is a bicistronic expression vector designed to express a target gene along with the puromycin selection marker (1). Upon transfection into a packaging cell line, this vector can transiently express, or integrate and stably express a viral genomic transcript containing the CMV immediate early promoter, gene of interest, IRES and the puromycin resistance gene (Pur^r). The gene of interest and the puromycin resistance gene are co-transcribed, via the internal ribosome entry site (IRES), as a bicistronic message in mammalian cells (2, 3).

This vector incorporates unique features including: optimization to remove promoter interference and self-inactivation. The hybrid 5' LTR consists of the cytomegalovirus (CMV) type I enhancer and the mouse sarcoma virus (MSV) promoter. This construct drives high levels of transcription in HEK 293-based packaging cell lines due, in part, to the presence of adenoviral E1A (4, 5, 6, 7) in these cells. The self-inactivating feature of the vector is provided by a deletion in the 3' LTR enhancer region (U3). During reverse transcription of the retroviral RNA, the inactivated 3' LTR is copied and replaces the 5' LTR, resulting in inactivation of the 5' LTR CMV enhancer sequences. This may reduce the phenomenon known as promoter interference (8) and allow more efficient expression.

Also included in the viral genomic transcript are the necessary viral RNA processing elements including the LTRs, packaging signal (Psi$^+$), and tRNA primer binding site. pQCXIP also contains a bacterial origin of replications and *E. coli* Amp^r gene for propagation and selection in bacteria.

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Use

pQCXIP is designed to deliver and express a gene along with the puromycin resistance marker from a bicistronic message. The design is optimized to produce high titers via the $P_{CMV\ IE}$ in the packaging cell line. The bicistronic transcript makes it unnecessary to screen the transformants since the puromycin resistance is expressed in concert with the gene inserted into the multiple cloning site.

Once transfected into the packaging cell line (such as the RetroPack™ PT67 Cell Line (Cat. No.631510) AmphoPack293, EcoPack2-293, or Pantropic System), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles since pQCXIP lacks structural genes (gag, pol, and env) necessary for particle formation and replication; however, these genes are stably integrated as part of the packaging cell genome. Once a high titer clone is selected, these retroviral particles can infect target cells and transmit the gene of interest but cannot replicate within these cells due to the absence of viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Location of Features

- 5' LTR (CMV/MSV): 1–728
  Cytomegalovirus (CMV)/ mouse sarcoma virus (MSV) hybrid promoter: 1–511
  R region: 584–654
  U5 region: 655–728
- Ψ^+ (extended packaging signal): 758–1567
- Immediate early CMV promoter ($P_{CMV\ IE}$): 1601–2132
- Multiple Cloning Site (MCS): 2239–2287
- Internal ribosome entry site (IRES): 2289-2862
- Puromycin resistance gene (Pur^r): 2898–3494
  Start codon (ATG): 2895–2897; stop codon (TGA): 3492–3494
- 3' MoMuLV LTR (deletion in U3): 3868–4293
  Poly A region: 4195–4216
- SV40 promoter: 4573–4840
- SV40 ori: 4794–4859
- Col E1 ori (Site of replication initiation): 5180
- Ampicillin resistance gene (β-lactamase): 6800–5940
  Start codon (ATG): 6800–6798 stop codon (TAA): 5940–5942

Sequencing Primer Locations

- pQC Seq/PCR Primers:
  5' primer (2141–2164): 5'-ACGCCATCCACGCTGTTTTGACCT-3'
  3' primer (2311–2334): 5'-AAGCGGCTTCGGCCAGTAACGTTA-3'

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

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Home : AdMax

Last Updated 1/07/09

AdMax

"We continue to have great success with the AdMax™ system for producing a wide range of vectors for our research. We can go from plasmid clone to high titre vector ready for in vitro or in vivo evaluation in just a few weeks."
Michael Parr Ph.D., Scientist, Gene Therapy/Delivery Group, Validation Biology, Biogen Inc.

"Our lab uses the AdMax™ system for construction of E1/E3-deleted Adenovirus vectors routinely, with excellent results. We have found this technology to be easy and reliable."
C.S.H. Young, Professor of Microbiology, Columbia University, New York, NY USA.

"This method works really great, it proved to be efficient and reliable. We think that so far this is the best available method for constructing the recombinant viruses."
Dr. Elena Burova, Associate Manager, Adenovirus Facility, Regeneron Pharmaceuticals, Inc.

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You will require the ACROBAT plug-in to view PDF files. If you do not have the plug-in, please download the latest version here.

Clone, cotransfect and GO!
Small shuttle plasmids, single cloning step, cotransfections without restriction, 95% reliability. The simplest, most efficient, most flexible system for construction of adenovirus expression vectors.

How fast?
How fast can you clone your gene into a small pUC based shuttle plasmid and prepare 100ug plasmid DNA? Add 7 to 10 days to that!

How efficient?
Approximately 100 fold more plaques rescued than with previous two plasmid methods.

How reliable?
If your expression cassette is less than 7-8 kb and your transgene product is nontoxic, 95% of recombinant viruses should contain and express the transgene. Use your favourite promoter or use the high efficiency MCMV IE promoter provided with our kits.

How simple?
Only two steps. No homologous recombination in difficult to handle bacterial systems; use your favourite bacterial strain. No transfer of candidate plasmids from one bacterial strain to another. No need for expensive, exotic restriction enzymes or for linearization of plasmid DNA prior to cotransfection of 293 cells. The system does not require lambda packaging or yeast technologies that are not standard procedures in the majority of labs.

How flexible?
Cassettes can be inserted in E1 or E3 or transgenes can be cloned into both regions. For example a transactivator can be inserted in E3 and a regulated expression cassette in E1. Vectors can be designed with an E3 deletion, a wild type E3 region or, if the transgene in E1 is small, a stuffer sequence can be inserted in E3 to prevent formation of RCA. You have a choice of two site specific recombinases: Cre or FLP, with similar high rescue efficiencies.

How expensive?
The initial cost of our kits is competitive with other systems, but unlike other kits ours allow for an infinite number of vector rescues. If you can grow plasmid DNA there is no need to purchase our kits more than once.

Back to Top

System Overview

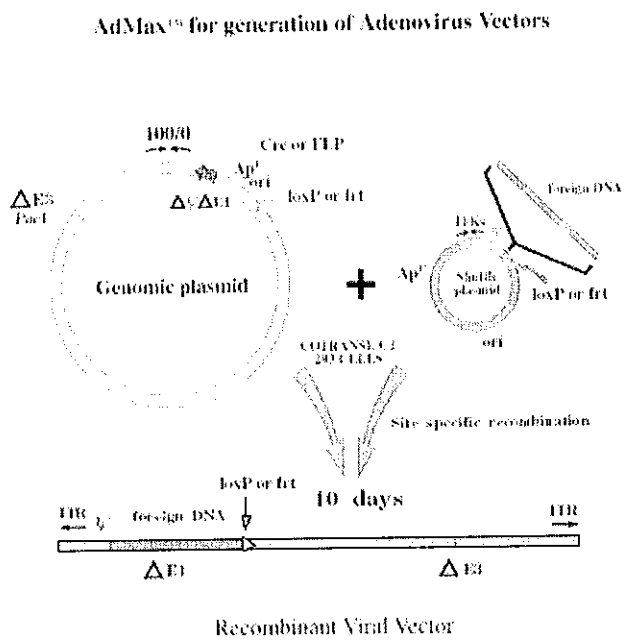

Figure 1 outlines the principles of the AdMax™ system with Cre-lox as an example. Recombination in cotransfected cells introduces the gene of interest into infectious Ad DNA while simultaneously excising the recombinase gene (Ng et al., 1999, 2000).

Neither the small shuttle plasmid nor the genomic plasmid need be digested with restriction enzymes prior to cotransfection. Any E1 complementing cell line such as 293 cells (Graham et al., 1977), 911 cells (Fallaux et al., 1996) or PERC6 cells (Fallaux et al., 1998) can be used for cotransfections.

Although rescue of viral vectors is highly efficient (over 100 fold greater than with the original two plasmid method of Bett et al., (1994)), and 95% of viruses generated by cotransfection should carry the transgene, it is good laboratory practice to build up working stocks of virus from plaque isolates before extensive experimentation.

Microbix provides low passage 293 cells that are especially cultured to maintain the strong adherence and plaque forming properties of the original 293 cells. For rapid production of vectors to be used in preliminary experiments, it may be possible to produce recombinant viruses by incubating cell cultures under liquid medium following cotransfections.

Transgenes are cloned into one of our small high copy number shuttle plasmids (Figures 2 and 4) which are then cotransfected with an Ad genomic plasmid (Figures 3 and 5) into 293 cells. High efficiency site specific recombination catalyzed by Cre or FLP recombinase results in "rescue" of the expression cassette into the left end of an E1 deleted (first generation) Ad vector.

Back to Top

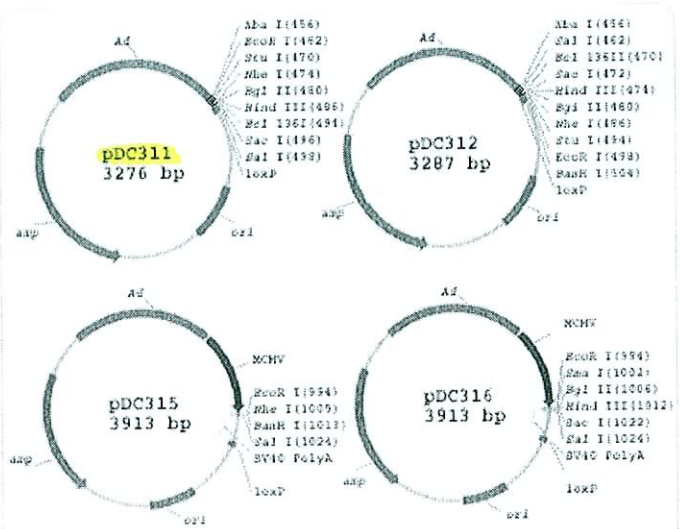

Figure 2. Shuttle plasmids for Cre-lox Ad vector construction

Shuttle plasmids **(Figure 2)** designed for insertion of the transgene are small, simple and pUC based for high yields. Promoterless plasmids with polycloning sites comprising recognition sites for 8 enzymes are only 3.2 kb in size. Plasmids containing an expression cassette utilizing the Murine Cytomegalovirus Immediate Early Gene promoter (MCMV Pr) are only 3.9 kb and have up to 6 restriction enzyme cloning sites. The genomic plasmids containing most of the Ad genome plus cassettes expressing recombinase and carrying the recombinase recognition site are approximately 34 kb in size. Two recombination systems are available, based on Cre-lox or FLP-frt.

Back to Top

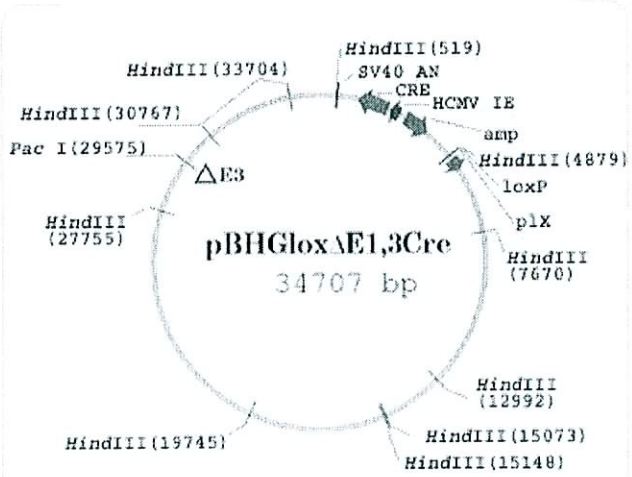

Figure 3. Ad genomic plasmid for construction of Ad vector by Cre-lox recombination

Figure 3 shows an example of one of the available Ad genomic plasmids containing a Cre expression cassette (which is excised during recombination with the shuttle plasmid). This plasmid can be purified and aliquoted and stored frozen for multiple vector rescue cotransfections. As little as 2 ug DNA/dish suffices to generate numerous plaques following cotransfection of 293 cells with a shuttle plasmid.

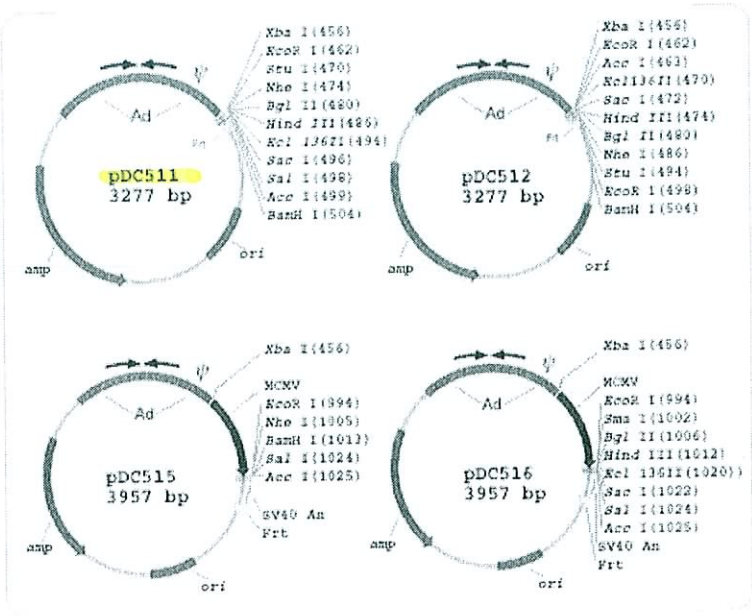

Figure 4 illustrates a set of shuttle plasmids analogous to those shown in Figure 2 but containing frt sites for recombination by the site specific recombinase, FLP, encoded by the yeast 2u plasmid (O'Gorman et al. Science 251, 1351, 1991).

Back to Top

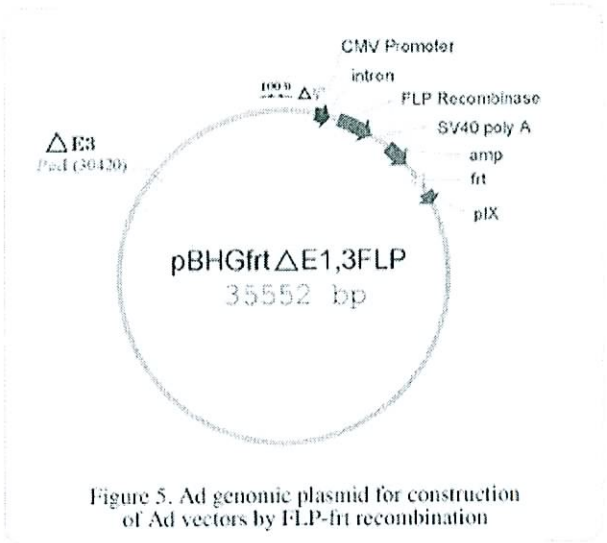

Figure 5. Ad genomic plasmid for construction of Ad vectors by FLP-frt recombination

The genomic plasmid encoding FLP and carrying an frt site for FLP mediated recombination with the shuttle plasmids of Figure 4 is illustrated in Figure 5. FLP functions as efficiently as Cre for production of adenovirus recombinants by site specific recombination between two cotransfected plasmids (Ng., et al., submitted). Plasmids can be propagated in any of the common bacterial strains such as DH5 alpha.

For recombinant DNA cloning any commonly used protocols will suffice but it is recommended that plasmid DNA to be used in cotransfections be prepared using the protocol provided with the kits.

Also we recommend that the simple cotransfection protocol provided with the kits be followed as closely as possible at least initially. Once the users have successfully rescued a number of transgenes and feel comfortable with the system, they are invited to try other plasmid DNA purification protocols and transfection methods.

For beginners we recommend that initial transfections be done using pFG140 (Graham, 1984), an infectious Ad genomic plasmid that serves as a positive control and which is provided free with all kits.

Because the only restriction enzymes required with the AdMax™ system are common enzymes used for cloning into the small

shuttle plasmids the AdMax™ system is simpler and more economical than methods requiring rare cutters (Chartier et al., 1996; He et al., 1998; Mizuguchi & Kay, 1998).

Moreover those rescue protocols typically use enzymes such as Pac I or SwaI to linearize plasmid DNA prior to transfection. If the transgene contains these sites then these methods are not practical. PacI sites, for example, are found surprisingly often in eukaryotic DNA. (There is one PacI site in the Murine Cytomegalovirus Immediate Early Gene promoter (one of the strongest viral promoters available (Addison et al., 1997)) and one also in the gene encoding luciferase, a popular reporter gene.)

The E3 deleted genomic plasmids contain a unique PacI cloning site in E3. It is possible to insert a reporter gene (Parks et al., 1996) or a gene for a transactivator in the E3 region to create a modified genomic plasmid that can then be combined with cassettes inserted in the E1 shuttle plasmid. Thus, for example, a series of vectors expressing genes under regulation by tet or by RU486 can be readily constructed using the AdMax™ system.

Back to Top

Ordering Information

AdMax™ Kits Available	
Catalogue#	**Microbix Product**
PD-01-64	Kit D (contains pDC311, pDC312, pDC315, pDC316, pBHGloxΔE1,3Cre, and pFG140)
PD-01-65	Kit E (contains pDC511, pDC512, pDC515, pDC516, pBHGfrtΔE1,3FLP, and pFG140)
PD-01-67	Kit F (contains pDC411, pDC412, pDC415, pDC416, pBHG10, pBHGE3 and pFG140)

AdMax™ Plasmids must be ordered in complete kits. Each plasmid is priced at 10 ug per vial.

Back to Top

Individual AdMax™ Plasmids

Catalogue#	**Microbix Product**
PD-01-29	pDC411
PD-01-30	pDC412
PD-01-31	pDC415
PD-01-32	pDC416

AdMax™ is covered by US patents 7,132,290; 6,855,534; 6,756,226; and 6,379,943

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Back to Top
