

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 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 _____
SIGNATURE Ross Feldman
DEPARTMENT Vasculature Biology
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PHONE NUMBER x 23928
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Location of experimental work to be carried out: Building(s) RRI Room(s) 4274

*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: HSFO
GRANT TITLE(S): Rapid Vascular Effect of Steroids

* 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:
QingMing Ding
Jozef Chorazymowski

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? YES NO
 If YES, please give the name of the species. _____
 What is the origin of the microorganism(s)? _____
 Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.
 Please describe any CFIA permit conditions:

1.2 Please complete the table below:

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
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>both</i>	Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>primary</i>	
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	HEK 293	
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Rat Vascular Smooth Muscle	
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No	Rat Endothelial cells	
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	Human blood	<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH52 JM109	PDC315 316	Microbi'X Bio tech	ACS, AC6 MR GPR30	

* 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 NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
adenovirus	adeno MR, GFP20, GFP, Sh1A	Vector Biolabs	AcS, AC6 MR, GFP, GFP20	

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

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

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? 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? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES 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? YES NO
If NO, please forward the permit to the 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? 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? YES NO

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

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ 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.

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

GRANT SUMMARY

Aldosterone and other vasoactive steroids are important physiological and pathophysiological regulators of cardiovascular function. The traditional view of the cardiovascular actions of the vasoactive steroids, like aldosterone, estrogen and testosterone has focused on their roles as regulators of transcription via activation of their "classical" receptors (Androgen Receptors -AR, Mineralocorticoid Receptors -MR and Estrogen Receptors -ER). However, based on a series of observations going back more than half a century, scientists have speculated that a range of steroids, including estrogens and aldosterone, might have effects on smooth muscle to regulate both vascular tone and cell growth and differentiation mediated by signalling mechanisms (like MAP kinase activation) that are too rapid to be accounted for by transcriptional regulation. Studies performed in our laboratory over the past several years have begun to elucidate the mechanisms by which steroids regulate peripheral resistance by rapid pathways.

At the single cell level, in vascular smooth muscle cells, aldosterone and estrogen both demonstrate rapid regulation of cell contraction/myosin light chain phosphorylation and regulation of MAP kinases. Further, we have most recently demonstrated that GPR30- a newly appreciated "orphan receptor" that has been implicated as the receptor mediating the rapid effects of estrogen, "promiscuously" mediates the rapid effects of both estradiol and aldosterone- as assessed at the single cell level. Further, we have shown that GPR30 expression reverses the effects of estradiol on ERK activation and apoptosis, to parallel the profile of aldosterone's rapid actions.

Based on these findings we now propose a series of studies to assess the role of GPR30 (vs. the role of ER and MR) in mediating the rapid effects of aldosterone and estrogen in vascular smooth muscle cells, vascular endothelial cells, and cardiac fibroblasts. All of this studies will be performed under control condition using adenovirus-expressing GFP,ER,MR or GPR30. These studies will be critical in understanding the overall cardiovascular effects of these steroids at a cellular level.

As noted above some of the common effects of aldosterone and estrogen are mediated by activation of GPR30. However, our initial studies were performed in isolated vascular smooth muscle cell systems. Whether this mechanism is important in the physiological regulation of vascular responses in whole organ systems or in vivo is unknown and is an important focus of this application.

Additionally we propose to elucidate the mechanism underlying the rapid vascular effects of testosterone. Previous studies have reported inconsistent effects of testosterone on rapidly-mediated vascular function that are vascular bed-/species-dependent. We hypothesize that this inconsistency may be related to testosterone mediating its rapid effects through multiple receptors- including GPR30. Thus we propose a series of studies to elucidate thereceptor-mediated mechanism underlying the rapid vascular effects of testosterone.

Lastly, we will determine whether GPR30-mediated responses (as well as ER-, AR and MR-mediated responses) are altered in hypertension.

These proposed studies, utilizing a range of integrative, cellular techniques and molecular techniques, will be important in understanding the mechanism of the rapid regulation of vascular function by aldosterone, estrogen and testosterone and may be critical in the development of new therapeutic approaches to modulate these pathways.

Generation of adenoviral constructs. Adenoviral constructs were generated with AdMax™ adenovirus vector creation kit as per manufacturer's instructions (Vector Biolabs). Briefly, GFP (used as control infections), MR or GPR30 cDNA were generated by PCR using plasmid templates of GFP cDNA (Clontech), hGPR30 (ATCC) or hMR (kindly provided by Dr Marc Lombes, INSERM, Paris, France). Resultant cDNAs were subcloned into shuttle vector pDC316 and purified. The recombinant plasmid was then co-transfected into Human Embryonic Kidney (HEK) 293 cells with adenoviral DNA pBHGlox (delta) E1, 3Cre. Recombinant adenovirus was harvested by lysis of transfected HEK293 cells using 3 freeze/thaw cycles. **The supernatant is used as source of adenovirus . We don't purified the adenovirus. Pease find attached MSDS sheet.**

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

only one available
no update

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045

<http://www.vectorbiolabs.com>

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:

All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5

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

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include: rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.

HOST RANGE: Humans and animals

INCUBATION PERIOD: from 1-10 days

MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection

CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.

PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.

SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT:

Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.

Inhalation: N/A

Ingestion: Wash out mouth with water. Call a physician

Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.

Wipe up carefully.

Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

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

PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:

Access to the laboratory is limited.

Work surfaces are decontaminated before and after each procedure

Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.

Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.

Laboratory coats are worn in and are removed before leaving the laboratory.

Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization

STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

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

Date of revision: May 24, 2004



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Cell Biology

ATCC® Number: **CRL-1573™** [Order this Item](#)

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Designations: 293 [HEK-293]

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Depositors: FL Graham

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Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]

[Cell Micrograph](#)

Shipped: frozen

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Medium & Serum: [See Propagation](#)

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Growth Properties: adherent

[Material Transfer Agreement](#)

Organism: *Homo sapiens* (human)

[Technical Support](#)

Morphology: epithelial

[Related Cell Culture Products](#)



PHOTO

Source: **Organ:** embryonic kidney
Cell Type: transformed with adenovirus 5 DNA

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

Restrictions: These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications: efficacy testing [[92587](#)]
transfection host ([Nucleofection technology from Lonza](#)
[Roche FuGENE® Transfection Reagents](#))
virucide testing [[92579](#)]

Receptors: vitronectin, expressed

Tumorigenic: Yes

DNA Profile (STR): Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9

D7S820: 11,12
 THO1: 7,9.3
 TPOX: 11
 vWA: 16,19

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

Age: fetus

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 segment 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 10³ to 6 X 10³ viable cells/cm² is recommended.
6. Incubate cultures at 37°C. Subculture when cell concentration is between 6 and 7 X 10⁴ cells/cm².

Subcultivation Ratio: 1:10 to 1:20 weekly.

Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

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

References:

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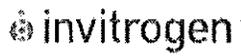
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ElectroMAX™ DH5 α -E™ Cells
Cat. No. 11319-019

ElectroMAX™ DH5 α -E™ cells are derived from the DH5 α ™ strain and are suitable for transformation by electroporation. They may be used in procedures requiring high transformation efficiencies, such as generation of cDNA

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

Product code 54357
Product name pUC 19 Control DNA

Company/Undertaking Identification

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

INVITROGEN CORPORATION
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

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

3. HAZARDS IDENTIFICATION

Ingestion No information available

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

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

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety ;

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

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

U.S. Federal Regulations

SARA 313

This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contain HAPs.

U.S. State Regulations

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

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

16. OTHER INFORMATION

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

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End of Safety Data Sheet



ElectroMAX™ DH5 α -E™ Cells
Cat. No. 11319-019

ElectroMAX™ DH5 α -E™ cells are derived from the DH5 α ™ strain and are suitable for transformation by electroporation. They may be used in procedures requiring high transformation efficiencies, such as generation of cDNA

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Home : AdMax

Last Updated 1/07/06

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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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.

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System Overview

AdMax™ for generation of Adenovirus Vectors

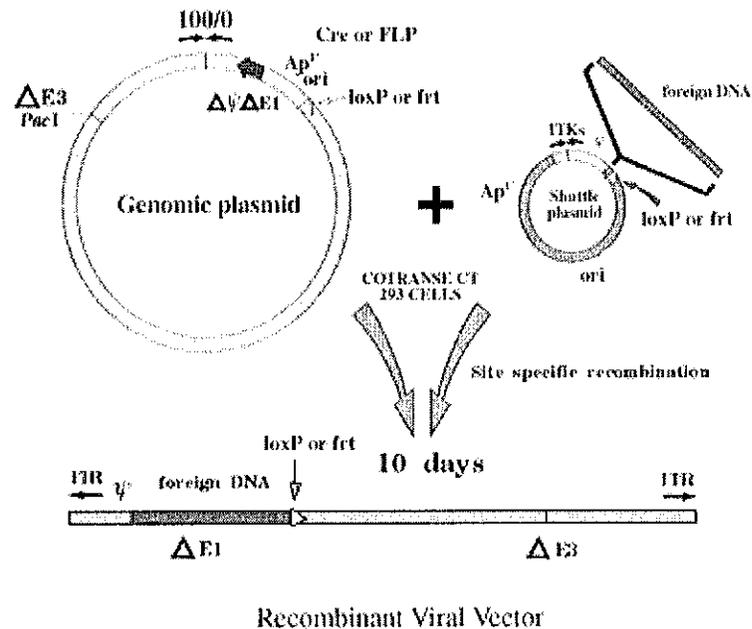


Figure 1 outlines the principles of the AdMax™ system with Cre-lox as an example. Recombination in cotransfected cells introduces the gene of interest into infectious Ad DNA while simultaneously excising the recombinase gene (Ng et al., 1999, 2000).

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.

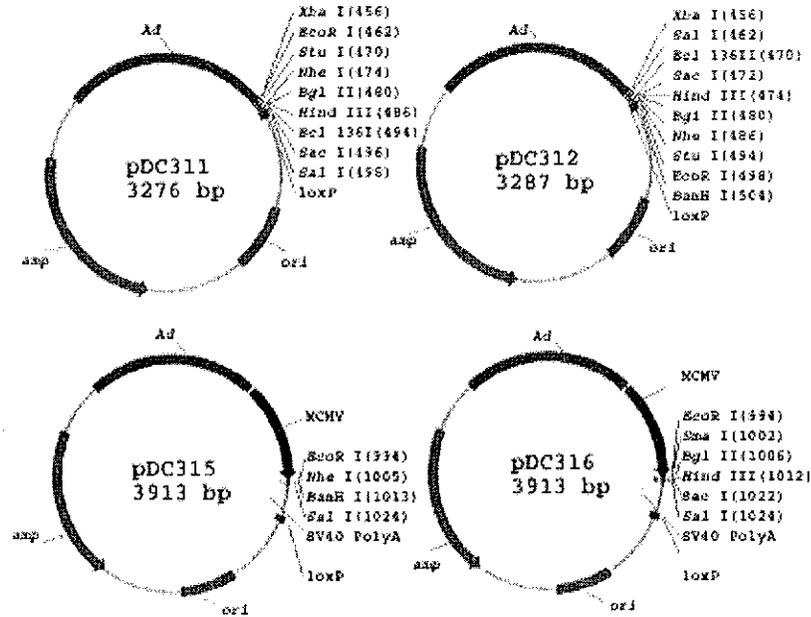
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Figure 2. Shuttle plasmids for Cre-lox Ad vector construction

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*.

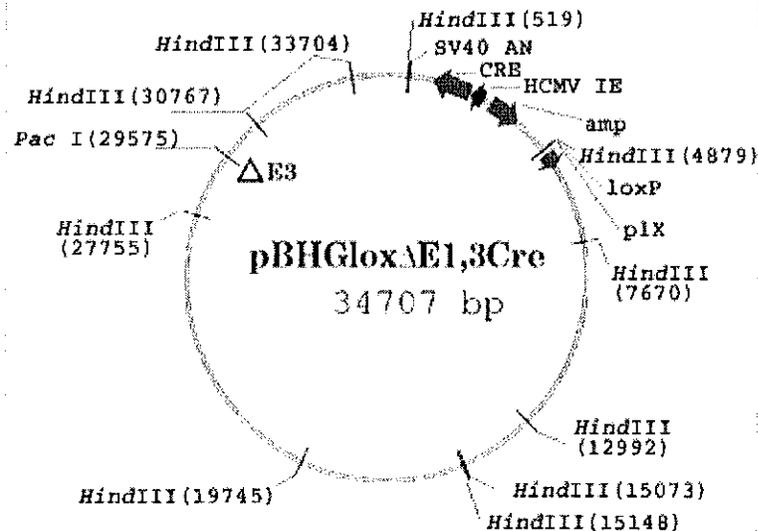
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Figure 3. Ad genomic plasmid for construction of Ad vector by Cre-lox recombination

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 SmaI 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.

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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.

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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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