The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

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

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

PRINCIPAL INVESTIGATOR:	**Robert A. Hegele**
DEPARTMENT:	**RRI VBRG**
ADDRESS:	**Room 4288A RRI**
PHONE NUMBER:	**519-931-5271**
EMERGENCY PHONE NUMBER(S):	**519-931-5271**
EMAIL:	**hegele@robarts.ca**

Location of experimental work to be carried out :

Building : **RRI** Room(s): **4288, 4292, 4286, 4284**

Building : _____ Room(s): _____

Building : _____ Room(s): _____

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR, HSFO**

GRANT TITLE(S): _____

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Jian Wang	**jwang@robarts.ca**	**30/08/11**
Henian Cao	**hcao@robarts.ca**	**30/08/11**
Adam McIntyre	**amcintyre@robarts.ca**	**24/03/09**
Brooke Kennedy	**bkennedy@robarts.ca**	**09/12/09**
Matthew Ban	**mban@robarts.ca**	**30/08/11**
Joseph Dube	**jdube5@uwo.ca**	**26/04/09**

Mary Bammimore	mbammimore@uwo.ca	30/09/10
John Robinson	robinson@robarts.ca	pending

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

My laboratory is focused on elucidating the genetic determinants of cardiometabolic syndromes in humans. As such, from consented research subjects, blood, saliva or buccal swabs are collected and genomic DNA or RNA is extracted. The DNA is then further assayed for candidate gene variation analysis using Sanger sequencing, microarrays or high throughput sequencing. The RNA is assayed using microarrays, q-pcr assays Sanger sequencing or potentially, high throughput (next-generation) sequencing.

Variations in sequences are then statistically tested for association with specific cardiometabolic phenotypes. If there is association, cell based assays where variant sequences are introduced through standard transfection protocols into cell lines and further genomic or western assays are performed. Sometimes immunofluorescence studies are performed to evaluate differences between variant and normal sequences in our candidate genes of interest. We typically use commercial reagents for transfection and mutagenesis studies.

There are cases where certain tissues are acquired and these tissues (currently brain and adipose tissues are registered on this permit) are processed to extract DNA or RNA and similar studies (as above) are performed. We also acquire cell lines from ATCC and Coriell Cell Repositories relevant to my research and similar treatment is given to the DNA and RNA.

All staff follow UWO procedure: "Laboratory procedures to be followed when handling unfixed human blood, tissues or body fluids (HBBF)". Human source material is transported in appropriate containment for UN3373: Biological Substance, Category B materials. Containers are opened in a certified tissue culture HEPA filtered hood and universal precautions are observed during the DNA or RNA extraction process. Primary biological materials are stored in a lockable refrigerator in my lab and DNA or RNA aliquots as well as plasma aliquots are stored either at 4° or -80°. Cell lines are stored in a LN2 Dewar. There are after hours staff contact labels on all of these storage devices.

Disposal of materials follows best practices for disposal of biological material. Waste liquid fractions are contained in small tubes and are delivered to the autoclave waste stream. Any disposable transfer plastics (pipette tips, serological pipettes) are similarly decontaminated. Surfaces are wiped clean with a 10% bleach solution and a 70% ethanol solution is used for final decontamination. Any centrifugation procedures occur in buckets fitted with biosafety containment systems which are bleach decontaminated after use.

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

My laboratory is focused on elucidating the genetic determinants of cardiometabolic syndromes in humans. As such, from consented research subjects, blood, saliva or buccal swabs are collected and genomic DNA or RNA is extracted. The DNA is then further assayed for candidate gene variation analysis using Sanger sequencing, microarrays or high throughput sequencing. The RNA is assayed using microarrays, q-pcr assays Sanger sequencing or potentially, high throughput (next-generation) sequencing.

Variations in sequences are then statistically tested for association with specific cardiometabolic phenotypes. If there is association, cell based assays where variant sequences are introduced through standard transfection protocols into cell lines and further genomic or western assays are performed. Sometimes immunofluorescence studies are performed to evaluate differences between variant and normal sequences in our candidate genes of interest.

There are cases where certain tissues are acquired and these tissues (currently brain and adipose tissues are registered on this permit) are processed to extract DNA or RNA and similar studies (as above) are performed. In some cases, we will harvest skin fibroblast tissue from research subjects to culture primary cell lines for further mutagenesis studies or immunohistochemistry assays or immunofluorescence studies.We also acquire cell lines from ATCC and Coriell Cell Repositories relevant to my research and similar treatment is given to the DNA and RNA.

1.0 Microorganisms

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

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

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions: **N/A**

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli DH10B	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.007 -0.010**	**LifeTechnologies**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
E. coli DH5A	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.007 -0.010**	**LifeTechnologies**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

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

Additional Comments: ____________________

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

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

Canada

2.0 Cell Culture

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

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

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**See attached**		**See attached**
Rodent	☒ Yes ☐ No	**See attached**		**ATCC**
Non-human primate	☒ Yes ☐ No	**COS7**		**ATCC**
Other (specify)	☐ Yes ☒ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

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

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
E. coli DH5A	**pcDNA3.1 CAT; pCMV6; pcDNA-Dest53 Gateway**	**Life Technologies**	**APOE, TMPRSS4**	**none**	**no**	**none**

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

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

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

5.0 Will genetic sequences from the following be involved?

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

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

Additional Comments: _____

6.0 Human Gene Therapy Trials

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

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

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

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

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

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

8.0 Use of Animal species with Zoonotic Hazards

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

8.2 Will live animals be used? ☐ YES ☐ NO

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

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

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

9.0 Biological Toxins and Hormones

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

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

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

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

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

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

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

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

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

11.3 What is the origin of the plant?

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

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

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

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

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

12.0 Import Requirements

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

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

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

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

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Robert A. Hegele ***Date:*** 14/02/12

14.0 Containment Levels

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

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

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-RRI-0006**

15.0 Procedures to be Followed

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

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Needlestick: encourage bleeding to flush wound, flush with water. bandage, seek first aid, report to OHS. Splash: PPE is to be worn, remove contaminated PPE, flush with water, seek first aid, report to OHS.

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Robert A. Hegele ***Date:*** 14/02/12

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not ~~UWO~~):
SIGNATURE: Ronald Hozkatt
Date: March 21, 2012

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

Special Conditions of Approval:

2012 Biohazard Agents Registry, Dr. Robert Hegele

29-Feb-12

Name	CAT. NO.	SUPPLIER	MSDS	Comment	Use
3T3-L1	CL-173	ATCC	Y	Murine fibroblast	Mutant LMNA is transfected into these cells and immunofluorescence studies and Western analysis performed
NIH/3T3	CRL-1658	ATCC	Y	Murine	
COS-7: note SV40 promoter	CRL-1651	ATCC	Y	Green monkey (Ceropithecus aethiops)	
HEK293: note Adeno E1A promoter	CRL-1573	ATCC	Y	Human	
HepG2	CRL-11997	ATCC	Y	Human	
Fibroblast, finite primary cell line human	GM05659	Coriell Cell Repository	Y	Human: unaffected	Cells are cultured, morphology and growth curves evaluated and immunofluorescence studies, Western analysis, DNA and RNA isolated and microarray and high throughput sequencing analysis performed.
Fibroblast, finite primary cell line human	GM08398	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	GM03348	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG03513	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG04456	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG16409	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	HGADFN167	Progeria Research Foundation	Y	Human: HGPS proband	same use as Coriell Cell Repository
Fibroblast, finite primary cell line human	30950	Dr. T.C. Rupar	na	Human	Cells are cultured, DNA and RNA extracted and microarray and high throughtput sequencing analysis performed
Fibroblast, finite primary cell line human	40916	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	20750	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	70280	Dr. T.C. Rupar	na	Human	
pCMV6	PS100001	Origene	Y		Transfection vectors used in the lab
pcDNA3.1	350492	Invitrogen	Y		
Subcloning Efficiency™ DH5a™ Competent Cells	18265-017	Invitrogen	Y	E. Coli	Cells used to as vehicles for transfection of genetic material for overexpression studies and phenotypic characterization
Electromax DH10B competent cells	18290-015	Invitrogen		E. Coli	
ME DH10B competent cells	18297-010	Invitrogen		E. Coli	
ME DH5-alpha competent cells	18258-012	Invitrogen	Y	E. Coli	
ME DH5A T1 page resist comp cells	12034-013	Invitrogen		E. Coli	
GFP-ICK-wt plasmid: pcDNA-DEST53 Gateway vector	12288-015	Invitrogen		E. Coli	
ICK ORF clone	ID: IOH38087	Invitrogen		E. Coli	

Cell Biology

ATCC® Number: **CL-173™** Order this Item

Price: **$431.00 (for-profit list price)**
$359.17 (non-profit list price)
Log In with customer # to see your price

See New Benefits of ATCC Culture

##Caption##

Designations:	3T3-L1
Depositors:	Massachusetts Institute of Technology
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	fibroblast 
Source:	**Organ:** embryo **Cell Type:** fibroblast
Cellular Products:	triglycerides
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
Receptors:	insulin, expressed
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

Related Links

NCBI Entrez Search
Cell Micrograph
Chemically-induced Differentiation
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement New!
Technical Support
Related Cell Culture Products
Product Information Sheet

Cell Biology

ATCC® Number:	**CRL-1658™** Order this Item	Price:	**$431.00 (for-profit list price)** **$359.17 (non-profit list price)** **Log In with customer # to see your price**

See New Benefits of ATCC Culture

Designations:	NIH/3T3
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	fibroblast 
Source:	**Organ:** embryo **Strain:** NIH/Swiss **Cell Type:** fibroblast
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
Virus Susceptibility:	Murine leukemia virus
Age:	embryo
Comments:	The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457]. Tested and found negative for ectromelia virus (mousepox).

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement New!

Technical Support

Related Cell Culture Products

Product Information Sheet

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

Cell Biology

ATCC® Number: **HB-8065™** Order this Item

Price: **$431.00 (for-profit list price)**
$359.17 (non-profit list price)
Log In with customer # to see your price

See New Benefits of ATCC Culture

Designations:	Hep G2
Depositors:	Wistar Institute
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein)
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
Receptors:	insulin; insulin-like growth factor II (IGF II) [22446]
Tumorigenic:	No

DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 F13A01: 5,7 F13B: 6,10

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement New!

Technical Support

Related Cell Culture Products

Product Information Sheet

BioProducts

- Cell, microbial and molecular genomics products for the life sciences

BioServices

- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

ATCC®

Product Information

Info on Cell Line(s)

Cell Line Designation: COS-7
ATCC® Catalog No. CRL-1651™

Table of Contents:

Cell Line Description

Organism: *Cercopithecus aethiops* (monkey, African green)
Tissue: kidney; SV40 transformed
Morphology: fibroblast
Growth Properties: adherent
Virus Susceptibility: SV40 (lytic growth); SV40 tsA209 at 40ºC; SV40 mutants with deletions in the early region
Depositor: Y. Gluzman
Comments: This 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.

Parental cell line -- ATCC® CCL-70™

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.

4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. *It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure For Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a

5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; 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 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 10 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.

   Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended.

6. Incubate cultures at 37°C.

   Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in ***Culture of Animal Cells: A Manual of Basic Technique*** by R. Ian Freshney, 4th edition, published by Wiley - Liss, N.Y., 2000.

Medium Renewal

Every 2 to 3 days

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%

This medium is formulated for use with a 5% CO_2 in air atmosphere. . (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 in air atmosphere is then recommended).

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500mL) or ATCC® Catalog No. 30-2021 (100mL) .

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

Additional references are available in the catalog at www.atcc.org

Gluzman Y. **SV40-transformed simian cells support the replication of early SV40 mutants**. Cell 23: 175-182, 1981 PubMed: 81162716

Fernandez LM and 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: 96132827

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: 96149362

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: 96386576

Gonzalez Armas JC et al. **DNA immunization confers protection against murine cytomegalovirus infection**. J. Virol. 70: 7921-7928, 1996 PubMed: 97048074

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: 96394543

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: 96215003

Lee JH et al. **The proximal promoter of the human transglutaminase 3 gene**. J. Biol. Chem. 271: 4561-4568, 1996 PubMed: 96224044

Chen Y et al. **Demonstration of binding of dengue virus envelope protein to target cells**. J. Virol. 70: 8765-8772, 1996 PubMed: 97126081

Russell DW and Miller AD. **Foamy virus vectors**. J. Virol. 70: 217-222, 1996 PubMed: 96099433

Wright DA et al. **Association of human fas (CD95) with a ubiquitin-conjugating enzyme (UBC-FAP)**. J. Biol. Chem. 49: 31037-31043, 1996 PubMed: 97094863

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: 96324889

Ozcelebi F et al. **Phosphorylation of cholecystokinin receptors expressed on chinese hamster ovary cells**. J. Biol. Chem. 271: 3750-3755, 1996 PubMed: 96216481

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: 96215142

Shaul PW et al. **Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae**. J. Biol. Chem. 271: 6518-6522, 1996 PubMed: 96198122

Ladner RD et al. **Identification of a consensus cyclin-dependent kinase phosphorylation site unique to the nuclear form of human deoxyuridine triphosphate nucleotidohydrolase**. J. Biol. Chem. 271: 7752-7757, 1996 PubMed: 96205968

Wu X et al. **Demonstration of a physical interaction between microsomal triglyceride transfer protein and apolipoprotein B during the assembly of ApoB-containing lipoproteins. J**. Biol. Chem. 271: 10277-10281, 1996 PubMed: 96215326

Hawes BE et al. **Phosphatidylinositol 3-kinase is an early intermediate in the G beta gamma-mediated mitogen-activated protein kinase signaling pathway**. J. Biol. Chem. 271: 12133-12136, 1996 PubMed: 96218120

Arai H and Charo IF. **Differential regulation of G-protein-mediated signaling by chemokine receptors**. J. Biol. Chem. 271: 21814-21819, 1996 PubMed: 96355570

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: 96291890

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: 96278987

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods **11**:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

07/07

Cell Line Designation: 293 (HEK293)
ATCC® Catalog No. CRL-1573™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: kidney; transformed with adenovirus 5 DNA
Age: fetus
Morphology: epithelial
Growth properties: adherent
Doubling time: about 19 hours
Tumorigenic: tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells.
Receptors expressed: vitronectin
Virus susceptibility: human adenoviruses
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13
D5S818: 8,9
D7S820: 11,12
TH01: 7,9.3
TPOX: 11
vWA: 16,19

Depositors: F.L. Graham
Comments: Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome, it is now clear that only left end sequences are present. The line is excellent for titrating human adenoviruses.
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.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit.
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).

Karyotype: 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.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA: from this line is available as ATCC Catalog No. CRL-1573D™ (10 µg).

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at −70°C. Storage at −70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the

contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended ifusing the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.
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 37°C.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Add 2.0 to 3.0 ml of 0.25% (w/v) Trypsin-0.53mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 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.

3. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.

4. 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/cm^2 is recommended.
   Subcultivation Ratio: 1:6 to 1:10 weekly

5. Incubate cultures at 37°C.

6. Subculture when cell concentration is between 6 and 7 X 10^4 cells/cm^2.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Two to three times weekly

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%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Da Costa LT, et al. **Converting cancer genes into killer genes.** Proc. Natl. Acad. Sci. USA 93: 4192-4196, 1996 PubMed: 96210616

Graham FL et al. **Characteristics of a human cell line transformed by DNA from human adenovirus type 5.** J. Gen. Virol. 36: 59-72, 1977 PubMed: 77229557

Graham FL et al. **Defective transforming capacity of adenovirus type 5 host-range mutants.** Virology 86: 10-21, 1978 PubMed: 78205587

Harrison T et al. **Host-range mutants of adenovirus type 5 defective for growth in HeLa cells.** Virology 77: 319-329, 1977 PubMed: 77129592

Bodary SC and McLean JW. **The integrin beta 1 subunit associates with the vitronectin receptor alpha v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line.** J. Biol. Chem. 265: 5938-5941, 1990 PubMed: 90202850

Goodrum FD and Ornelles DA. **The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle.** J. Virol. 71: 548-561, 1997 PubMed: 97138357

Loffler S et al. **CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus.** J. Virol. 71: 42-49, 1997 PubMed: 97138295

Hu SX et al. **Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression.** Cancer Res. 57: 3339-3343, 1997 PubMed: 97413605

Kolanus W et al. **alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule.** Cell 86: 233-242, 1996 PubMed: 96319726

Stauderman KA et al. **Characterization of human recombinant neuronal nicotinic acetylcholine receptor subunit combinations alpha 2 beta 4, alpha 3 beta 4 and alpha 4 beta 4 stably expressed in HEK293 cells.** J. Pharmacol. Exp. Ther. 284: 777-789, 1998 PubMed: 98122961

Bartz SR et al. **Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control.** J. Virol. 70: 2324-2331, 1996 PubMed: 96183878

Sandri-Goldin RM and Hibbard MK. **The herpes simplex virus type 1 regulatory protein ICP27 coimmunoprecipitates with anti-sm antiserum, and the C terminus appears to be required for this interaction.** J. Virol. 70: 108-118, 1996 PubMed: 96099420

Ansieau S et al. **Tumor necrosis factor receptor-associated factor (TRAF)-1, TRAF-2, and TRAF-3 interact in vivo with the CD30 cytoplasmic domain; TRAF-2 mediates CD30-induced nuclear factor kappa B activation.** Proc. Natl. Acad. Sci. USA 93: 14053-14058, 1996 PubMed: 97098519

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: 96324889

Oppermann M et al. **Monoclonal antibodies reveal receptor specificity among G-protein-coupled receptor kinases.** Proc. Natl. Acad. Sci. USA 93: 7649-7654, 1996 PubMed: 96353872

Xia Y et al. **Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury.** Proc. Natl. Acad. Sci. USA 93: 6770-6774, 1996 PubMed: 96270618

Zhu X et al. **Cell cycle-dependent modulation of telomerase activity in tumor cells.** Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996 PubMed: 96234095

Uebele VN et al. **Functional differences in Kv1.5 currents expressed in mammalian cell lines are due to the presence of endogenous Kvbeta2.1 subunits.** J. Biol. Chem. 271: 2406-2412, 1996 PubMed: 96161969

Abell A et al. **Deletions of portions of the extracellular loops of the lutropin/choriogonadotropin receptor decrease the binding affinity for ovine luteinizing hormone, but not human choriogonadotropin, by preventing the formation of mature cell surface receptor.** J. Biol. Chem. 271: 4518-4527, 1996 PubMed: 96224039

Tiberi M et al. **Differential regulation of dopamine D1A receptor responsiveness by various G protein-coupled receptor kinases.** J. Biol. Chem. 271: 3771-3778, 1996 PubMed: 96216484

Shahrestanifar M et al. **Studies on inhibition of mu and delta opioid receptor binding by dithiothreitol and N-ethylmaleimide. His223 is critical for mu opioid receptor binding and inactivation by N-ethylmaleimide.** J. Biol. Chem. 271: 5505-5512, 1996 PubMed: 96215005

Boring L et al. **Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1alpha receptors.** J. Biol. Chem. 271: 7551-7558, 1996 PubMed: 96205938

Noonberg SB et al. **Evidence of post-transcriptional regulation of U6 small nuclear RNA.** J. Biol. Chem. 271: 10477-10481, 1996 PubMed: 96209965

Fox JC and Shanley JR. **Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells.** J. Biol. Chem. 271: 12578-12584, 1996 PubMed: 96218185

Lee MJ et al. **The inducible G protein-coupled receptor edg-1 signals via the Gi/mitogen-activated protein kinase pathway.** J. Biol. Chem. 271: 11272-11279, 1996 PubMed: 96212194

Marchand P et al. **Cysteine mutations in the MAM domain result in monomeric meprin and alter stability and activity of the proteinase.** J. Biol. Chem. 271: 24236-24241, 1996 PubMed: 96394562

Arai H and Charo IF. **Differential regulation of G-protein-mediated signaling by chemokine receptors.** J. Biol. Chem. 271: 21814-21819, 1996 PubMed: 96355570

Huang Q et al. **Substrate recognition by tissue factor-factor VIIa.** J. Biol. Chem. 271: 21752-21757, 1996 PubMed: 96355561

Monteclaro FS and Charo IF. **The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1alpha receptor, confers chemokine selectivity.** J. Biol. Chem. 271: 19084-19092, 1996 PubMed: 96325007

Keith DE et al. **Morphine activates opioid receptors without causing their rapid internalization.** J. Biol. Chem. 271: 19021-19024, 1996 PubMed: 96324996

Louis N et al. **Cloning and sequencing of the cellular-viral junctions from thehuman adenovirus type 5 transformed 293 cell line.** Virology 233: 423-429, 1997 PubMed: 97360037

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines.** 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

06/11

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

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015, Rm 4-25
100 Perth Drive
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell line from donor with Hutchinson-Gilford Progeria Syndrome (HGADFN167), as provided by The Progeria Research Foundation Cell Bank, 532 Lowell Street, Peabody, MA, USA, 01960.

Marianne Heisz
Chief, Importation and Regulatory Affairs

JULY 16, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

The Progeria Research Foundation, Inc

DECLARATION STATEMENT

The contents of this package are as follows:

Cultured Human dermal fibroblasts specimen in medium containing 15% fetal bovine serum (certified free of infectious agents) in a sealed collection tube.

These samples are considered to be non-infectious and are for research purposes only.

These samples are being shipped from Dr. Leslie Gordon and Dr. Douglas Hixson for studies funded by The Progeria Research Foundation. Samples are packed in approved blood mailers and are perishable. Samples ***are not*** known to be infectious. **Please do not delay**.

If you have any questions or concerns, please contact Dr. Leslie Gordon, Principal Investigator, at the following phone number: (508) 889-6655
Sincerely,

Leslie B. Gordon, MD, PhD

P. O. Box 3453, Peabody, MA 01961-3453
Tel: (978) 535-2594, Fax: (978) 535-5849,
Email: info@progeriaresearch.org

www.progeriaresearch.org

The Progeria Research Foundation
Cell and Tissue Bank

October 19th, 2009

Dear Dr. Robinson,
Blackburn Cardiovascular Genetics Laboratory
Robarts Research Institute
Room 4-28, PO box 5015, 100 Perth Drive
London, On, Canada, N6A 5K8

Please find enclosed one flask each of the following cell lines which you requested from the PRF Cell and Tissue Bank for your research.

Cell Line #	Passage #	Clinically Affected?	Relation to Proband	Age at Donation	Exon 11 mutation Yes or no C→T
HGADFN167	4	yes	proband	8 yrs. 5mos	yes

Please place cells at 37°C and 5% CO_2 for 24 hours upon receipt and then change the culture medium and split (if necessary) as directed. Please see the attached sheet containing specific culture conditions. If you have any further questions do not hesitate to contact me at leslie_gordon@brown.edu or Lorraine Fast (Laboratory Technician) at 401-444-7564 or lfast1@lifespan.org.

Sincerely,

Leslie B. Gordon, MD, PhD
Principal Investigator, The Progeria Research Foundation Cell and Tissue Bank

Email: progeria@netzero.net Website: www.progeriaresearch.org
P. O. Box 3453, Peabody, MA 01961-3453 Tel: (978) 535-2594 Fax: (978) 535-5849

Culture Conditions:
DMEM (Gibco 11960-044), + 2mM L-Glutamine, Pen/Strep and 15% FBS.

Split Conditions:
0.25% Trypsin/ EDTA - 1ml/T25 flask, evenly coating cells. Incubate 2-3 minutes. Gently tip flask to dislodge. Pool cells in culture medium and replate or freeze down.

Freezing Conditions:
10% DMSO in culture media

Email: progeria@netzero.net Website: www.progeriaresearch.org
P. O. Box 3453, Peabody, MA 01961-3453 Tel: (978) 535-2594 Fax: (978) 535-5849

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

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015
100 Perth Drive, Rm 4-25
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell line from healthy donor (AG16409), as provided by Coriell Institute for Medical Research, 403 Haddon Avenue, Camden, NJ, USA 08103.

Marianne Heisz
Chief, Importation and Regulatory Affairs

JULY 16, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

Catalog ID: **AG16409**

Product (Source): CELL CULTURE

ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Unspecified
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 12 YR
Sex Male
Race Caucasian
Family 1936
Family Member 1
Relation to Proband proband
Clinically Affected No
Confirmation Clinical summary/Case history
ISCN 46,XY
Remarks The donor was clinically normal having suffered a cervical spine injury at age 5. He was ventilator-dependent. He died of brain death with cardiorespiratory arrest at age 12. The culture was initiated on 7/12/2000 using explants of minced skin tissue taken post-mortem. The cell morphology is fibroblast-like. The karyotype is 46,XY with 4% of the cells examined showing random chromosome loss and 2% showing random chromosomal aberrations.

Catalog ID AG16409
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
How to Order Online Ordering
Assurance Form (Must have current form on file)
Statement of Research Intent Form (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
PDL at Freeze 4
Passage Frozen 2

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Nucleoside Phosphorylase,Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase Isoenzyme Electrophoresis

Phenotypic Data

Remark The donor was clinically normal having suffered a cervical spine injury at age 5. He was ventilator-dependent. He died of brain death with cardiorespiratory arrest at age 12. The culture was initiated on 7/12/2000 using explants of minced skin tissue taken post-mortem. The cell morphology is fibroblast-like. The karyotype is 46,XY with 4% of the cells examined showing random chromosome loss and 2% showing random chromosomal aberrations.

Publications

Data are not available

External Links

dbSNP dbSNP ID: 11159

Images

Data are not available

Protocols

PDL at Freeze	4
Passage Frozen	2
Split Ratio	1:4
Temperature	37 C
Percent CO2	5%
Medium	Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids
Serum	10% fetal bovine serum Not inactivated
Substrate	None specified
Subcultivation Method	trypsin-EDTA

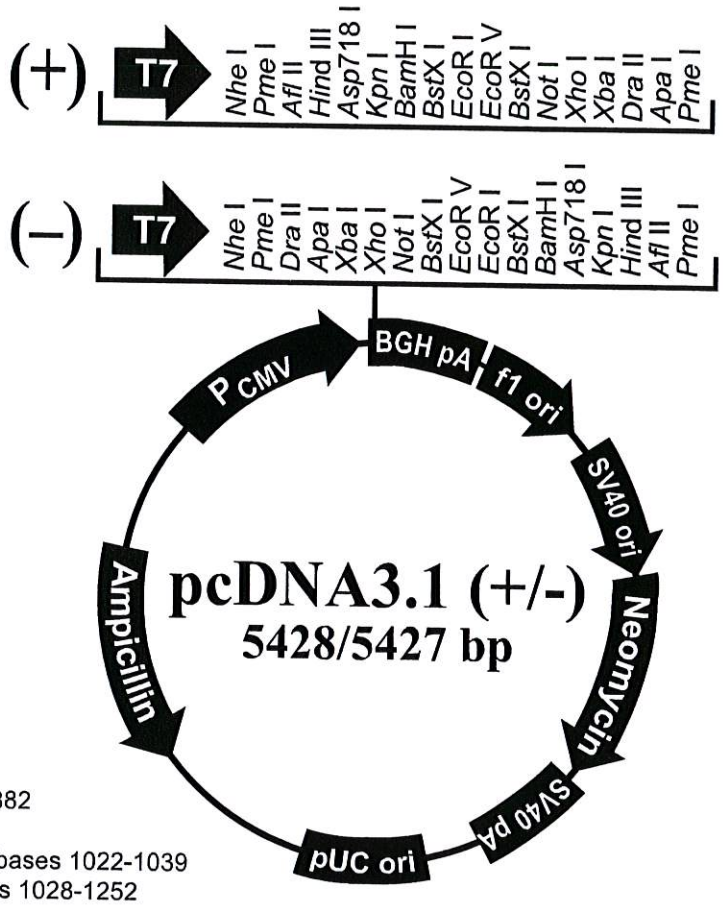
Appen

Section 4.2

pcDNA3.1 Vectors

Map of pcDNA3.1(+) and pcDNA3.1(-)

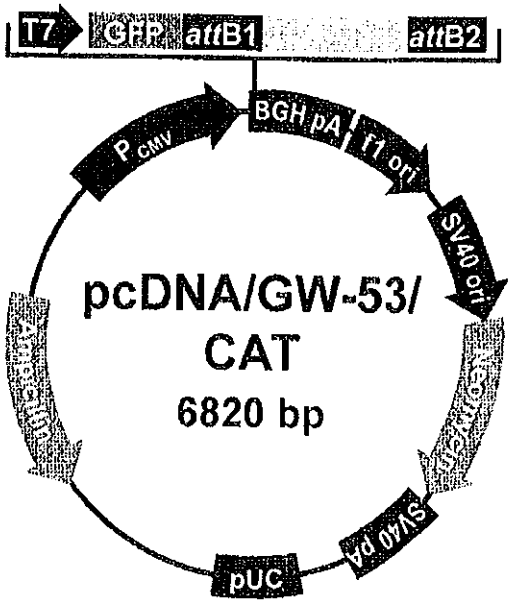
The figure below summarizes the features of the pcDNA3.1(+) and pcDNA3.1(-) vectors. **The complete sequences for pcDNA3.1(+) and pcDNA3.1(-) are available for downloading from our World Wide Web site** (www.invitrogen.com) **or from Technical Service (see page 13).** Details of the multiple cloning sites are shown on page 3 for pcDNA3.1(+) and page 4 for pcDNA3.1(-).



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

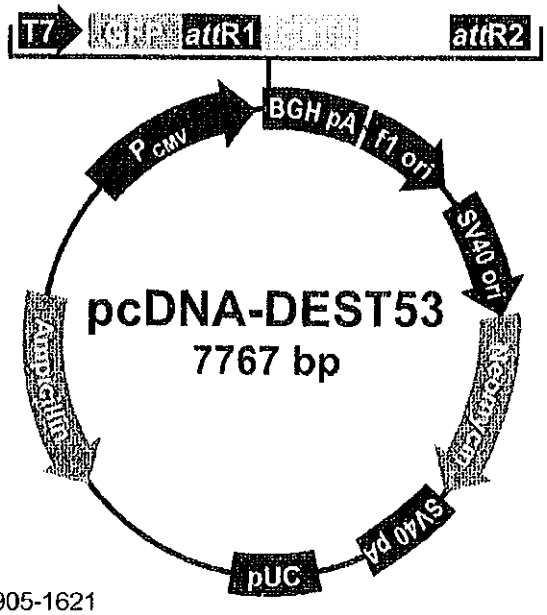
continued on next page



Comments for pcDNA/GW-53/CAT
6820 nucleotides

CMV promoter: bases 232-819
T7 promoter: bases 863-882
Cycle 3 GFP (N-terminal): bases 905-1621
*att*B1 recombination site: bases 1643-1667
CAT ORF: bases 1697-2353
*att*B2 recombination site: bases 2355-2379
BGH polyadenylation region: bases 2414-2641
f1 origin: bases 2687-3115
SV40 early promoter and origin: bases 3142-3450
Neomycin resistance ORF: bases 3525-4319
SV40 early polyadenylation region: bases 4493-4623
pUC origin: bases 5006-5679
Ampicillin resistance ORF (*bla*): bases 5824-6684 (c)
bla promoter: bases 6685-6783 (c)
(c) = complementary strand

Invitrogen™
life technologies



Comments for pcDNA-DEST53
7767 nucleotides

CMV promoter: bases 232-819
T7 promoter: bases 863-882
Cycle 3 GFP (N-terminal): bases 905-1621
*att*R1 recombination site: bases 1643-1767
Chloramphenicol resistance gene: bases 1876-2535
*ccd*B gene: bases 2856-3161
*att*R2 recombination site: bases 3202-3326
BGH polyadenylation region: bases 3361-3588
f1 origin: bases 3634-4062
SV40 early promoter and origin: bases 4089-4397
Neomycin resistance ORF: bases 4472-5266
SV40 early polyadenylation region: bases 5440-5570
pUC origin: bases 5953-6626
Ampicillin resistance ORF (*bla*): bases 6771-7631 (c)
bla promoter: bases 7632-7730 (c)
(c) = complementary strand

Invitrogen™
life technologies

MATERIAL SAFETY DATA SHEET

Revised 8/26/03
Replaces (None)
Printed 8/26/03

PCDNA-DEST53 (GATEWAY VECTOR)
INVITROGEN CORPORATION
MSDS ID: 12288

1. PRODUCT AND COMPANY INFORMATION

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

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

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

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

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

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

Product Name: PCDNA-DEST53 (GATEWAY VECTOR)
Stock Number: 12288

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

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

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

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

Component	CAS No.	Percent
EDTA	60-00-4	1 - 5
TRIZMA BASE	MIXTURE	60 - 100

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if swallowed.
Harmful if absorbed.
Harmful by inhalation.
May cause allergic skin reaction.
Possible reproductive system hazard based on animal data.
****

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

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

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.
Harmful! Can cause systemic damage (see "Target Organs).

Ingestion:
Mildly irritating to mouth, throat, and stomach. Can cause abdominal discomfort.
Harmful if swallowed. May cause systemic poisoning.

Chronic:
No data on cancer.
Contains a substance that is a possible reproductive system hazard based on animal studies at doses that could be encountered in the workplace.

4. FIRST AID MEASURES

Eye:
Immediately flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention and monitor the eye daily as advised by your physician.

Skin:
Wash with soap and water. Remove contaminated clothing, launder

4. FIRST AID MEASURES (CONT.)

immediately, and discard contaminated leather goods. Get medical attention immediately.

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

Ingestion:
Severely irritating. Do not induce vomiting. Seek medical attention immediately. Drink 2 glasses of water or milk to dilute.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.
Use water spray/fog for cooling.

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

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

6. ACCIDENTAL RELEASE MEASURES

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

6. ACCIDENTAL RELEASE MEASURES (CONT.)

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill.
Ventilate the contaminated area.
Prevent the spread of any spill to minimize harm to human health and the environment if safe to do so. Wear complete and proper personal protective equipment following the recommendation of Section VIII at a minimum. Dike with suitable absorbent material like granulated clay. Gather and store in a sealed container pending a waste disposal evaluation.

7. HANDLING AND STORAGE

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

Storage Pressure:
Ambient

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

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

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
EDTA	Not established.	Not established.
TRIZMA BASE	Not established.	Not established.

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

Personal Protective Equipment:

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

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

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

Respiratory:
NIOSH approved air purifying respirator with dust/mist filter. A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. High temperatures. Strong alkalies. Copper alloys. Aluminum alloys.

10. STABILITY AND REACTIVITY (CONT.)

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Nitrogen oxides.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
Not determined.

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
TRIZMA BASE: 5900 MG/KG

Target Organs: Kidneys. Bone marrow.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

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

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

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

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

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

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

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
EDTA	D2A
TRIZMA BASE	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
Not applicable.

15. REGULATORY INFORMATION (CONT.)

PRODUCT CLASSIFICATION:
XI

Component	EINECS Number
EDTA	200-449-4
TRIZMA BASE	Not established.

16. OTHER INFORMATION

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

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

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

* The ORFCard has links to several external public sites for your convenience.
Those links may be occasionally unavailable

Ultimate™ ORFCard for Clone ID IOH38087

Gene Information

Clone ID:	IOH38087
Organism:	*Homo sapiens*
Matching Nucleotide Accession:	NM_014920.3|Alignment
Related Accession(s):	AL031178|Alignment || AL162581|Alignment || AB023153|Alignment || AF152469|Alignment || AF225919|Alignment || AI699136|Alignment || AJ420557|Alignment || AK074892|Alignment || BC035807|Alignment || BX647493|Alignment
Gene Name:	intestinal cell (MAK-like) kinase
Gene Definition:	Homo sapiens, intestinal cell (MAK-like) kinase (ICK), transcript variant 1, mRNA
Gene Symbol:	ICK
Summary:	Eukaryotic protein kinases are enzymes that belong to a very extensive family of proteins which share a conserved catalytic core common with both serine/threonine and tyrosine protein kinases. This gene encodes an intestinal serine/threonine kinase harboring a dual phosphorylation site found in mitogen-activating protein (MAP) kinases. The protein localizes to the intestinal crypt region and is thought to be important in intestinal epithelial cell proliferation and differentiation. Alternative splicing has been observed at this focus and two variants, encoding the same isoform, have been identified.
Expression:	Sage Tag Expression || Virtual Northern || Digital Expression Profile
Transcript Variant 1:	This variant (1) represents the shorter transcript.
Transcript Variant 2:	This variant (2) has an additional exon in the 5' UTR, as compared to variant 1. Variants 1 and 2 encode the same isoform.
mRNA Record:	NM_014920|Alignment || NM_016513|Alignment
GO Category: biological process	development (GO:0007275) protein amino acid phosphorylation (GO:0006468) protein kinase cascade (GO:0007243) signal transduction (GO:0007165)
GO Category: molecular function	ATP binding (GO:0005524) magnesium ion binding (GO:0000287) protein serine/threonine kinase activity (GO:0004674) transferase activity (GO:0016740)
References:	GRIF: 22858 | PUBMED: ICK

ORF Information

ORF length (bp):	1899
Sequence:	Nucleotide || Peptide || Translation || Quality Scores || Quality Scores with Sequence

Clone Information

Collection Name:	Ultimate ORF Clones
Collection Type:	ORF Gateway™ Entry
Vector Name:	pENTR(tm)221
Vector Antibiotic:	Kanamycin
Host Name:	E.coli

Protein

Protein Accession:	CAI20261|Alignment || CAI19518|Alignment || BAA76780|Alignment || AAG43364|Alignment || AAF37278|Alignment || AAH35807|Alignment || Q9UPZ9|Alignment
Protein Record:	NP_055735|Alignment || NP_057597|Alignment
Physical Properties:	(aa) || 0.0 (MW) || 0.0 (pI)
Protease Digestion:	Trypsin | Lys-C | Arg-C | Asp-N | V8-bicarb | V8-phosph | Chymotrypsin | CNBr
Predicted Secondary Structure:	View Secondary Structure
Protein Model Search:	Swiss-Model BLAST
Product:	intestinal cell kinase

SNP Information

SNP:	All rs in gene region | rs in coding region only | rs with heterozygosity only
SNP Map to:	Protein

Genomic Link

LocusLink ID:	22858
Unigene ID:	Hs.417022
Genome Alignment:	Map to Human Genome using BLAT || Map to Ensembl Genome Browser

Quarterly, Invitrogen™ Corporation will update the Ultimate™ ORF Clone Collection, by adding, deleting, or modifying the bioinformatic information to more accurately reflect the most current scientific information associated with each of the clones represented in the collection.

It is highly recommended that the purchaser carefully evaluate the ORF clone sequence prior to purchase. It is the purchaser s responsibility to check whether the sequence associated with any particular clone meets his/her scientific requirement prior to purchase.

Invitrogen makes no warranties, express or implied, that the manufacture, use, importation, or sale of the gene contained within any Ultimate™ ORF clone will not infringe any third party intellectual property right.

Western

robarts RESEARCH

November 1, 2011

To: Mr. Ron Noseworthy & RRI/UWO Biosafety Committee

RE: Use of human source materials from clinical samples from patients and research subjects not from known risk groups.

Includes:

1. Peripheral blood, saliva, buccal swabs and primary skin fibroblast cell lines for DNA or RNA extraction as well as molecular cell biology and immunohistochemistry techniques.

Specific biosafety procedures.

1. All staff will follow UWO procedure: Laboratory procedures to be followed when handling unfixed human blood, tissues or body fluids (HBBF). Universal Biosafety level 2 precautions will be observed.
2. Blood, saliva, buccal cells and primary skin fibroblast cells have been added to Hegele BIO-RRI-0006 permit.
3. Tissue transport to Hegele lab: 1) Blood will be collected by venipuncture into plastic Becton-Dickinson EDTA tubes. Saliva and buccal swabs will be collected into Oragene (DNA Genotek Inc, Ottawa, ON) DNA collection kits OG-500 and OG-575 respectively. Any transport will have double containment in a sealed plastic bag, labelled with a biohazard label, with absorbent material sufficient to contain any liquid spill. Any transport other than locally by hand that is required will be in an appropriate container for UN3373 materials.2) Primary fibroblast cell lines will be transported to the Hegele lab in an appropriate container. Flask(s) of cells will be brought to Room 4292 and processed in the Level 2 culture facility.
4. DNA or RNA from blood leucocytes, saliva or buccal swabs will be extracted using Gentra PureGene kits, following the instructions for the kit. Instructions are attached for both DNA and RNA extraction.
5. All centrifugation will be carried out in a Beckman GS-6R centrifuge in 15 ml conical tubes inside a biosafety containment rotor.
6. Any waste (typically a pellet after lysis and proteinase treatment) will be treated with a fresh 1:10 bleach:water solution and then discarded into the autoclave waste stream. All surfaces and apparatus will be decontaminated with a fresh 1:10 bleach:water solution. After use, all plastic pipette tips, plastic vessels and tubes will be placed in the autoclave waste stream.

100 Perth Drive, Box 5015, London, ON N6A 5K8 Canada
TEL: 519-931-5271; FAX: 519-931-5218 www.robarts.ca

7. The resultant DNA will undergo DNA sequence analysis for candidate genes and RNA will be used for gene expression assays. DNA aliquots are stored in either a 4 degree fridge or at -80 and RNA aliquots will be stored at -80 for subsequent use.

Robert A. Hegele MD, FRCPC, FACP, FAHA, FCAHS
Jacob J. Wolfe Distinguished Medical Research Chair in Human Gene Function
Edith Schulich Vinet Canada Research Chair in Human Genetics
Martha G. Blackburn Chair in Cardiovascular Research
Scientist, Robarts Research Institute
Distinguished Professor of Medicine and Biochemistry
The University of Western Ontario
hegele@robarts.ca

Revision: #4 November 1, 2011

Western

robarts RESEARCH

October 20, 2010

To: Mr. Ron Noseworthy & RRI/UWO Biosafety Committee

RE: Use of human source materials from clinical samples from patients not from known risk groups.

Includes:

1. Collaborative research project with Dr. Michael Strong to sequence candidate genes in DNA from fresh, frozen human brain samples (normal controls, familial and sporadic ALS).
2. Collaborative research project with Dr. Rob Gros to sequence candidate genes in DNA and/or assay for gene expression in RNA from fresh or frozen human adipose tissue samples.

Specific biosafety procedures.

1. All staff will generally follow UWO procedure: Laboratory procedures to be followed when handling unfixed human blood, tissues or body fluids (HBBF). Universal level 2 precautions will be observed.
2. Brain tissue and adipose tissue has been added to Hegele BIO-RRI-0006 permit.
3. Tissue transport to Hegele lab: 1) Brain tissue will be transported from Dr. Strong's laboratory on the third floor of Robarts in 50 ml. conical tubes inside a sealed zip lock bag inside a freezer box. Box will be labelled with biohazard warning label and stored at -80 in Hegele freezer #3 in room 4295. Brain samples will be approximately 1 cm^3 each and will include normal, familial and sporadic ALS samples. 2) Adipose tissue will be transported from Dr. Gros's laboratory on the fourth floor of Robarts in 50 ml. conical tubes either frozen or at 4° then -80 in RNAlater solution. Box will be labelled with biohazard warning label and stored at -80 in Hegele freezer #3 in room 4295.
4. The 50 ml tubes will be transported to Hegele Level 2 Culture Suite (Room 4292) and thawed inside a Microzone HEPA filtered hood. Samples will be removed from the 50 ml conical tube and either homogenized in a Dounce homogenizer, a Qiagen TissueRuptor in Gentra PureGene lysis buffer solution using puncture proof gloves to hold the homogenizer or minced and manually processed in a plastic vessel in lysis solution. After homogenization or manual disruption, the solution will be transferred to plastic 15 ml conical tubes and DNA or RNA will be extracted as per the instructions on the kit. Instructions are attached for both DNA and RNA extraction.
5. All centrifugation will be carried out in a Beckman GS-6R centrifuge in 15 ml conical tubes inside a biosafety containment rotor.
6. Solid phase tissue waste (typically a pellet after lysis and proteinase treatment) will be treated with 1-2 drops Wescodyne and then discarded into the autoclave waste stream. All

100 Perth Drive, Box 5015, London, ON N6A 5K8 Canada
TEL: 519-931-5271; FAX: 519-931-5218 www.robarts.ca

surfaces and apparatus will be decontaminated with a fresh 1:10 bleach:water solution. We will autoclave the Dounce homogenizer between homogenizations. After use, all plastic pipette tips, plastic vessels and tubes will be placed in a fresh 1:10 bleach:water solution with a 30 minute residence time, then bleach solution will be decanted – after a water rinse, tips and tubes will be placed in the autoclave waste stream.

7. The resultant DNA will undergo DNA sequence analysis for candidate genes and RNA will be used for gene expression assays. DNA aliquots are stored in either a 4 degree fridge or at -80 for subsequent use and RNA will be stored at -80 for subsequent use.

Robert A. Hegele MD, FRCPC, FACP, FAHA, FCAHS
Jacob J. Wolfe Distinguished Medical Research Chair in Human Gene Function
Edith Schulich Vinet Canada Research Chair in Human Genetics
Martha G. Blackburn Chair in Cardiovascular Research
Scientist, Robarts Research Institute
Distinguished Professor of Medicine and Biochemistry
The University of Western Ontario
hegele@robarts.ca

Revision: #3 October 20, 2010