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:	**Nica Borradaile**
DEPARTMENT:	**Physiology and Pharmacology**
ADDRESS:	**DSB 2011**
PHONE NUMBER:	**82107**
EMERGENCY PHONE NUMBER(S):	**519 636 9683**
EMAIL:	**nica.borradaile@schulich.uwo.ca**

Location of experimental work to be carried out :

Building : **DSB** Room(s): **2011**
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, CDA**

GRANT TITLE(S): **Roles of elongation factor 1A-1 in apolipoprotein B metabolism and the pathogenesis of nonalcoholic fatty liver disease (CIHR); Protective effects of NAD+ on endothelial cell survival and angiogenesis during type 2 diabetes (CDA)**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): **N/A**

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

Name	UWO E-mail Address	Date of Biosafety Training
Alexandra Stoianov	**astoiano@uwo.ca**	**10/2010**
Jennifer Hughes-Large	**jhughesl@uwo.ca**	**11/2010**
Alexandra Hetherington	**ahether4@uwo.ca**	**05/2012**
Dominic Pang	**dpang8@uwo.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.

Bacterial plasmids and cells, and cultured human cells (primary and cell line) are used for biochemical, cell biological, and molecular biology experiments associated with both grant funded projects. Live cells are contained in carbon dioxide incuabators and biological safety cabinets, and stored long-term in liquid nitrogen. Upon completion of experiments, cells and biohazardous wastes are decontaminated in a solution of chlorine bleach (10%), and disposed into biohazard waste bags followed by autoclaving.

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

Roles of elongation factor 1A-1 in apolipoprotein B metabolism and the pathogenesis of nonalcoholic fatty liver disease (CIHR)

Obesity and adult onset (type 2) diabetes have become epidemic in North America and are associated with multiple organcomplications, including liver disease. Research suggests that the elevated blood lipids (fats) in obese diabetic individualsaccumulate in the liver causing dysfunction and/or death of the cells that make up this organ. This can lead to nonalcoholic fatty liver disease, a disease which has recently become a common cause of liver transplantation.

The liver of a healthy individual can export excess fat. However, clinical studies have shown that liver fat export is impairedin patients with nonalcoholic fatty liver disease. This is because of the increased breakdown of a protein critical for fat export from the liver, apoB. Another protein, eEF1A-1, which we have shown to be increased in cells exposed to excess fat, may be involved in this increased breakdown of apoB.

The studies associates with this project will use cellular and molecular biological techniques in cultured liver cells and in obese diabetic mice to determine whether increased eEF1A-1 protein levels in the liver contribute to increased apoB breakdown during nonalcoholic fatty liver disease. If this is the case, developing medications to inhibit eEF1A-1 in the liver might improve nonalcoholic fatty liver disease.

Protective effects of NAD+ on endothelial cell survival and angiogenesis during type 2 diabetes (CDA)

Blood vessel (vascular) diseases, including peripheral vascular disease, are common complications in patients with obesity and type 2 diabetes. Damage to endothelial cells, the cells which line all blood vessels, is caused by high blood glucose and lipid levels. Once endothelial cells are injured, the ability of blood vessels to repair further damage is limited, leading to the worsening of vascular disease.

Our objective is to investigate whether increasing the level of an important molecule involved in endothelial cell survival, NAD+, will improve the ability of blood vessels to repair the damage that occurs during obesity and type 2 diabetes.

We will study whether increasing NAD+ can improve endothelial cell survival during exposure to high blood lipids by using human endothelial cells grown in culture. We will also use an obese, diabetic mouse model of peripheral vascular disease to test whether increasing NAD+ can improve blood vessel repair. We will increase NAD+ levels by giving cultured cells and mice compounds which are precursors for NAD+ synthesis.

The studies assoicated with this project directly address the mission of the Canadian Diabetes Association. Since obesity and type 2 diabetes are on the rise in Canada, one of the biggest challenges to our health care system is the management of vascular complications in these patients. Developing new drug therapies to increase NAD+ and improve endothelial cell survival could reduce vascular disease in individuals with obesity and type 2 diabetes.

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:
No risk.

Please attach the CFIA permit.

Please describe any CFIA permit conditions:
None.

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 XL-1 Blue competent	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1L**	**Stratagene**	☒ 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
	☐ 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: ______________________

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	**Lonza (commercial source)**	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	**HepG2**	**1**	**ATCC**
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
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		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	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?
XL-1 Blue	**pSilencer 2.1-U6 hygro, pECE SIRT1 H363Y**	**Ambion, AddGene**	**eEF1A-1 siRNA, SIRT1 H363Y**	**Yes**	**No**	**stress resistance, stress intolerance**

**** 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 **Mouse**

7.3 AUS protocol # **2010-018; 2011-044**

7.4 List the location(s) for the animal experimentation and housing. **West Valley Building**

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 # **C-11-0478** ☐ 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 Nica Borradaile ***Date:*** July 17, 2012

14.0 Containment Levels

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

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

14.3 Please indicate permit number (not applicable for first time applicants): **UWO-BIO-0243**

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:
All required safety procedures will be followed, including immediate washing of affected injury area, and reporting to the ER and OHS as necessary.

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 Nica Borradaile ***Date:*** July 17, 2012

15.4 Additional Comments: _____

16.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

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

www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # TS-CC-37-8 07/11

Clonetics™ dermal microvascular endothelial cell systems

HMVEC-d & D-HMVEC-d

Introduction

Clonetics™ dermal microvascular endothelial cell systems contain dermal-derived normal human microvascular endothelial cells (HMVEC-d) or diseased human microvascular endothelial cells from diabetic donors (D-HMVEC-d) and optimized medium for their growth. Each system can quickly generate HMVEC-d or D-HMVEC-d cultures for experimental applications in oncology, wound healing, angiogenesis and drug development. Clonetics™ dermal microvascular endothelial cell systems are convenient and easy to use, allowing the researcher to focus on results. Cryopreserved HMVEC-d and D-HMVEC-d are shipped in third passage. Proliferating HMVEC-d and D-HMVEC-d are shipped in fourth or fifth passage.

Clonetics™ cells, medium and reagents are quality tested together and guaranteed to give optimum performance as a complete cell system.

Cell system components

- One dermal derived microvascular endothelial cell product (cryopreserved or proliferating).
- Clonetics™ EGM™-2-MV BulletKit™ (CC-3202) contains one 500 ml bottle of endothelial cell basal medium-2 and the following growth supplements: hEGF, 0.5 ml; hydrocortisone, 0.2 ml; GA-1000, 0.5 ml; FBS, 25 ml; VEGF, 0.5 ml; hFGF-B, 2.0 ml; R^3-IGF-1, 0.5 ml; ascorbic acid, 0.5 ml.
- One ReagentPack™ (CC-5034) containing:

Trypsin/EDTA	100 ml
Trypsin neutralizing solution	100 ml
HEPES buffered saline solution	100 ml

Characterization of Cells

Routine characterization of HMVEC-d and D-HMVEC-d includes immunofluorescent staining. Cells stain positive for acetylated LDL and von Willebrand's (factor VIII) antigen and stain negative for smooth muscle α-actin.

Performance

Recommended seeding density for subculture	5,000 cells/cm^2
Typical time from subculture to confluent monolayer	5 - 9 days
HMVEC-d additional population doublings guaranteed using Clonetics™ System	15
D-HMVEC-d additional population doublings guaranteed using Clonetics™ system	Tested through 3 passages for information only

Quality control

HIV-1, hepatitis B and hepatitis C are not detected for all donors and/or cell lots. All cells are performance assayed and test negative mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Clonetics™ media are formulated for optimal growth of specific types of normal human cells. A Certificates of analysis (COA) for each lot of cryopreserved cells is shipped with each order. COA for all other products are available upon request.

Ordering information

Cryopreserved cells

CC-2505	HMVEC-d-Neo, neonatal	≥ 500,000 cells
CC-2516	HMVEC-d-Neo, neonatal, pooled	≥ 500,000 cells
CC-2543	HMVEC-d-Ad, adult	≥ 500,000 cells
CC-2929	D-HMVEC-d adult diabetic type I	≥ 500,000 cells
CC-2930	D-HMVEC-d adult diabetic type II	≥ 500,000 cells

Proliferating cells – Flasks and multiwell plates

HMVEC-d-Neo, neonatal

CC-2605	T-25 flask
CC-0246	T-75 flask
CC-0112	96-well plate

HMVEC-d-Neo, neonatal, pooled

CC-2616	T-25 flask
CC-0288	T-75 flask
CC-2516W96	96-well plate

HMVEC-d-Ad, adult

CC-2643	T-25 flask
CC-0207	T-75 flask
CC-2543W96	96-well plate

Other proliferating formats are available. Contact Scientific Support or refer to the Lonza website for details.

CC-3202	EGM™-2MV BulletKit™, EBM™-2 plus SingleQuots™ of growth supplements	500 ml
CC-3156	EBM™-2, endothelial cell basal medium-2	500 ml
CC-4147	EGM™-2MV SingleQuots™, formulates EBM™-2 to EGM™-2MV	
CC-5034	ReagentPack™	
	Trypsin neutralizing solution	100 ml
	Trypsin/EDTA solution	100 ml
	HEPES buffered saline	100 ml

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

Product Warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza guarantees the performance of its cells only if Clonetics™ media and reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system. Cryopreserved HMVEC-d and D-HMVEC-d are assured to be viable and functional when thawed and maintained properly.

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

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing can not offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

Cell Biology

ATCC® Number: **HB-8065™** Order this Item Price: **$264.00**

Designations:	Hep G2
Depositors:	Wistar Institute
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
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; [3525] 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) [3525]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin; 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 FESFPS: 11 LPL: 10,11 THO1: 9 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	modal number = 55 (range = 50 to 60); has a rearranged chromosome 1 [3525]
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. [23557] The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to gramoxone (oxidative stress). [26594] There is no evidence of a Hepatitis B virus genome in this cell line. [1205] [22909]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

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Subculturing:

Protocol:

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

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: Twice per week

Preservation:

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

Related Products:

recommended serum:ATCC 30-2020
derivative:ATCC CRL-10741
derivative:ATCC CRL-11997
purified DNA:ATCC HB-8065D
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

References:

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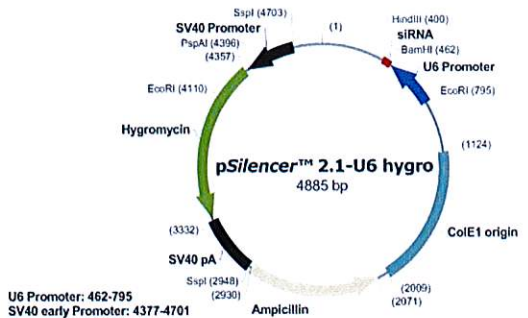
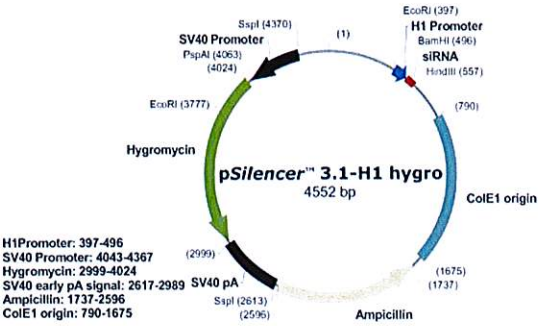
Return to Top

Figure 1. p*Silencer* hygro vector map

(These maps show the vectors containing typical siRNA template inserts.)



U6 Promoter: 462-795
SV40 early Promoter: 4377-4701
Hygromycin: 3332-4357
SV40 early pA signal: 2951-3323
Ampicillin: 2071-2930
ColE1 origin: 1124-2009



H1Promoter: 397-496
SV40 Promoter: 4043-4367
Hygromycin: 2999-4024
SV40 early pA signal: 2617-2989
Ampicillin: 1737-2596
ColE1 origin: 790-1675

C. siRNA Template Design

The prototypical siRNA comprises two hybridized 21-mer RNA molecules with 19 complementary nucleotides and 3' terminal dinucleotide overhangs. Expression vectors with dual promoters that express the two strands of the siRNA separately can be used (Lee 2002), however, a more efficient scheme is to express a single RNA that is a 19-mer hairpin with a loop and 3' terminal uridine tract (Paddison 2002) (Figure 2). When expressed in mammalian cells, the short hairpin siRNA can

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Plasmid 1792: Flag-SIRT1 H363Y

Gene/insert name:	SIRT1
Alternative names:	Sir2
Insert size (bp):	Unknown
Gene/insert aliases:	SIRT1, SIR2L1
Species of gene(s):	H. sapiens (human)
Relevant mutations/deletions:	H363Y, deacetylase domain mutation
Fusion proteins or tags:	Flag
Terminal:	N terminal on insert
Vector backbone:	pECE (Search Vector Database)
Type of vector:	Mammalian expression
Backbone size (bp):	2900
Cloning site 5':	HindIII
Site destroyed during cloning:	No
Cloning site 3':	XbaI
Site destroyed during cloning:	No
5' Sequencing primer:	SV40pro-F (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/1792
Author's Map:	Visit www.addgene.org/1792
Plasmid Provided In:	DH5a
Principal Investigator:	Michael Greenberg

Article: Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Brunet A et al. (Science 2004 Mar 26;303(5666):2011-5. Pubmed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

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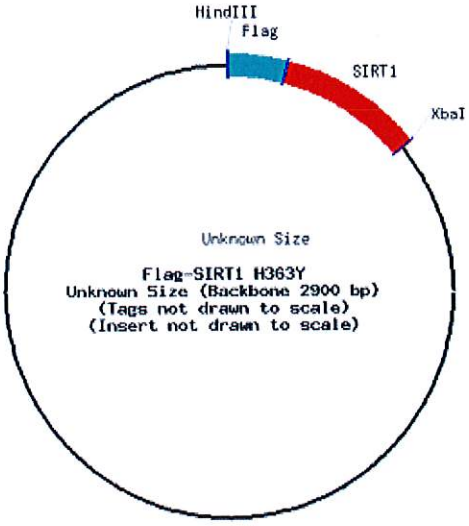

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Page 2 of 2 - Date: 08/31/2009