THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

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Location of experimental work to be carried out: Building(s) MSB Room(s) 235

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: None yet.
GRANT TITLE(S): See attached summary sheets.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:
None yet.

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

1.0 Microorganisms

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

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
XL1-Blue competent	O Yes Ø No	O Yes Ø No	O Yes Ø No	1L	Stratagene	Ø 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? Ø YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	Ø Yes O No	artery endothelium (from Lonza, see attached)	Not applicable
Rodent	Ø Yes O No	liver	None yet.
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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)*	Supplier / Source
Human	Ø Yes O No	HepG2	ATCC
Rodent	Ø Yes O No	McArdle RH-7777	ATCC
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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

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

3.0 Use of Human Source Materials

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

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

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

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
XL1-Blue competent	pSilencer 3.3-H1, pECE SIRT1 H363Y	Ambion, AddGene	eEF1A-1 siRNA, SIRT1 H363Y	stress resistance, stress intolerance

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mouse

6.3 AUS protocol # None yet. I'm working on these currently.

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ⊗ NO If no, please proceed to Section 9.0

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

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 Ø NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

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

SIGNATURE Marie Bouvadaile

13.0 Containment Levels

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

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

O YES, permit # if on-campus_____
☒ NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE Marie Bouvadaile Date: Sept. 1, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

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

Protective effects of NAD^+ on endothelial cell survival and regenerative angiogenic function during metabolic syndrome

Endothelial dysfunction is a major factor in the development of cardiovascular compli-cations, including atherosclerosis, and peripheral and cardiac ischemia, in patients with metabolic syndrome and type 2 diabetes mellitus (T2DM). In response to hyperglycemia and hyperlipidemia, endothelial cells (ECs) lose the ability to mediate vasodilation, become proinflammatory, and can eventually die. This limits the vascular repair and regenerative (postnatal) angiogenic functions of the endothelium. Excess glucose and fatty acids can trigger EC dysfunction and death through several mechanisms, but share the ability to in-crease cellular reactive oxygen species (ROS). In addition, excess fatty acids can be incorporated into endoplasmic reticulum (ER) membranes, resulting in ER stress. Both oxidative and ER stress can trigger EC apoptosis. Optimizing the cellular metabolism of excess glucose and fatty acids can channel these substrates away from pathways that generate oxidative and ER stress. We recently showed that increasing aerobic glycolysis in human aortic ECs during glucose overload promotes the use of excess glucose for angiogenesis, while limiting oxidative stress. In addition, increasing either mitochondrial beta-oxidation or stearoyl-CoA desaturase 1 (SCD1) expression during fatty acid overload prevents the incorporation of palmitate into ER membranes, and subsequent ER stress and apoptosis. Glycolysis, mitochondrial beta-oxidation, and SCD1 expression can all be regulated by the NAD^+-dependent protein deacetylase, SIRT1. In fact, overexpressing the rate-limiting enzyme for NAD^+ salvage synthesis in ECs enhances SIRT1 activity, conferring resistance to oxidative stress and improving angiogenic function during glucose overload. Cellular NAD^+ levels can also be augmented by supplementation with the precursors, nico-tinamide mononucleotide (NMN) and nicotinic acid (NA). Whether increasing NAD^+ synthesis can improve EC survival and enhance angiogenesis during fatty acid overload is unknown.

Hypothesis: Optimizing NAD^+ availability will enhance EC capacity for fatty acid β-oxidation by mitochondria and/or fatty acid storage in lipid droplets during fatty acid overload. This channeling of excess fatty acids away from pathways that lead to cell death will improve EC survival and angiogenesis during metabolic syndrome.

Objectives:

1. *Determine whether increasing cellular NAD^+ availability enhances EC metabolism of excess fatty acid, and improves EC survival and angiogenic function during fatty acid overload.* Human iliac artery ECs will be exposed to excess palmitate in the absence or presence of NMN or NA. Palmitate β-oxidation and storage as triglycerides will be assessed by metabolic labeling. EC survival will be determined through assays for oxidative stress, ER stress and apoptosis. In vitro angiogenic function will be assessed by tube formation on Matrigel.

2. *Determine whether NMN and NA promote SIRT1-dependent mitochondrial enzyme and SCD1 expression in ECs, thereby channeling excess fatty acids away from pathways leading to cell death.* ECs will be incubated with palmitate, NMN and NA, as in Aim 1. Expression of mitochondrial enzymes and SCD1 will be assessed by real time PCR and immunoblotting. EC survival will be assessed as in Aim 1. The involvement of SIRT1 in these effects will be determined by overexpression of a dominant-negative SIRT1 deacetylase mutant (SIRT1 H363Y).

3. *Determine whether increasing NAD^+ availability in a mouse model of diet-induced metabolic syndrome enhances vascular regeneration in areas of hind leg ischemia.* Metabolic syndrome will be induced in 129S6/SvEv mice by feeding western diet for 15 weeks. Control and metabolic syndrome mice will subsequently undergo unilateral femoral artery ligation, followed by treatment with vehicle, NMN or NA. Recovery of blood flow will be monitored by laser Doppler perfusion imaging.

Relevance: These studies will determine whether increasing cellular and systemic NAD^+ availability, through pharmacological means, can improve EC survival and enhance regenerative angiogenic capacity during the fatty acid overload that accompanies metabolic syndrome or T2DM. Therapeutic optimization of NAD^+ metabolism to improve endothelial function could reduce cardiovascular complications in patients with metabolic syndrome or T2DM.

Regulation of apolipoprotein B100 secretion by eukaryotic elongation factor 1A-1 during the development of nonalcoholic fatty liver disease

Obesity and type 2 diabetes mellitus (T2DM) are associated with excessive fatty acid and triglyceride accumulation in non-adipose tissues, such as the liver. Through the process of lipotoxicity, this ectopic lipid accumulation results in cellular dysfunction, cell death, and eventually organ dysfunction. The most common complication of obesity and T2DM, after cardiovascular disease, is nonalcoholic fatty liver disease (NAFLD). With a prevalence of about 65% in obese diabetic patients worldwide, NAFLD has become a common cause of liver transplantation. NAFLD encompasses a spectrum of liver disease ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), the lipotoxic consequence of progressive steatosis. Although the liver can export excess lipid via lipoprotein secretion, clinical studies indicate that apolipoprotein B100 (apoB) synthesis is decreased in patients with NASH. Moreover, recent studies in mice indicate that decreased apoB secretion in response to prolonged hepatic lipid overload is secondary to the induction of endoplasmic reticulum (ER) stress and subsequent increase in apoB degradation. Thus, ER stress resulting from hepatic lipid accumulation may impair apoB-containing lipoprotein secretion, increasing the potential for progression from simple steatosis to NASH.

In cultured hepatocytes, lipotoxicity is accompanied by rapid induction of ER stress and the unfolded protein response. In order to unload the ER of misfolded protein resulting from ER stress, the unfolded protein response stimulates ER-associated degradation via the 26S proteasome. This protein disposal mechanism also accounts for the majority of apoB degradation in hepatocytes. Interestingly, eukaryotic elongation factor 1A-1 (eEF1A-1), a critical mediator of lipotoxicity secondary to ER stress, is also involved in proteasome-mediated degradation of newly synthesized and damaged proteins. Furthermore, eEF1A-1 is partially localized to microsomes. Thus the rapid induction of eEF1A-1 which occurs in response to lipid overload may contribute to the ER stress-induced increase in apoB degradation observed in steatotic hepatocytes, resulting in impaired lipoprotein secretion and the progression of NAFLD.

Hypothesis: Excessive hepatic lipid accumulation induces ER stress in hepatocytes, triggering an increase in the ER-associated degradation of apoB which requires the expression of eEF1A-1. Moreover, hepatic eEF1A-1 expression is induced during obesity-associated NAFLD in vivo.

Objectives:

1. *Determine whether the ER stress-induced increase in apoB degradation that occurs in hepatocytes during lipid overload requires the expression of eEF1A-1.* Rat McA-RH7777 and HepG2 hepatoma clones with reduced eEF1A-1 expression will be generated by transfection with pSilencer (Ambion) constructs encoding hairpin siRNA templates. To assess apoB metabolism during the onset of lipid overload-induced ER stress, cells will be pre-incubated with medium containing palmitate. Cellular and secreted apoB synthesized in control and eEF1A-1 knockdown hepatoma cells, will be compared by pulse-chase with ^{35}S-methionine.

2. *Determine whether hepatic eEF1A-1 expression is induced during obesity-associated NAFLD in mice.* To determine whether hepatic eEF1A-1 is induced in conjunction with ER stress in a diet-induced model of obesity-associated NAFLD, C57BL/6 mice will be maintained on either control or high fat (Western) diet for 16 weeks. Livers will be harvested upon sacrifice and processed for histological analyses of steatosis, and mRNA and protein analyses of eEF1A-1 and markers of ER stress.

Summary: The goal of this proposal is determine whether induction of eEF1A-1 in response to lipid overload plays a role in the ER stress-induced increase in apoB degradation observed in steatotic hepatocytes. If this is the case, strategies to inhibit eEF1A-1 expression or activity during the development of NAFLD may preserve the ability of the liver to export excess lipid via lipoprotein secretion. This may prevent disease progression from simple steatosis to NASH.

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

Stratagene XL1-Blue Competent Cells, Catalog #200249

1 . Product and company identification

Product name	:	**Stratagene XL1-Blue Competent Cells, Catalog #200249**
Part No.	:	pUC18 Control Plasmid DNA 200231-42 1.42 M 2-Mercaptoethanol 210200-43 XL1-Blue Competent Cells 200236-41
Manufacturer / Supplier	:	Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Emergency telephone number	:	1-800-894-1304
Use of the substance/preparation	:	Chemical Kit
Validation date	:	01/09/2009

2 . Hazards identification

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		XL1-Blue Competent Cells	Liquid.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
OSHA/HCS status	:	pUC18 Control Plasmid DNA	While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
		1.42 M 2-Mercaptoethanol	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
		XL1-Blue Competent Cells	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
Emergency overview-Signal Word	:	WARNING !	
Emergency overview-Label Statement	:	pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
		1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
		XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

2 . Hazards identification

	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed. Irritating to eyes and skin. May cause sensitization by skin contact. Do not breathe vapor or mist. Do not ingest. Do not get on skin or clothing. Avoid contact with eyes. Wash thoroughly after handling.
	XL1-Blue Competent Cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling.
	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Routes of entry :	pUC18 Control Plasmid DNA	Eye contact. Ingestion.
	1.42 M 2-Mercaptoethanol	Dermal contact. Eye contact. Inhalation. Ingestion.
	XL1-Blue Competent Cells	Eye contact. Inhalation. Ingestion.
Potential acute health effects		
Eyes :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to eyes.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Skin :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed.
	XL1-Blue Competent Cells	Toxic if swallowed.
Medical conditions aggravated by over-exposure :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation.
	XL1-Blue Competent Cells	Repeated or prolonged exposure to the substance can produce target organs damage.
Over-exposure signs/symptoms :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
1.42 M 2-Mercaptoethanol		
2-Mercaptoethanol	60-24-2	10
XL1-Blue Competent Cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

Eye contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	:	pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.

4 . First aid measures

Ingestion	:	pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Notes to physician	:	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5 . Fire-fighting measures

Flammability of the product	:	pUC18 Control Plasmid DNA	Non-flammable.
		1.42 M 2-Mercaptoethanol	Non-flammable.
		XL1-Blue Competent Cells	Non-flammable.
Products of combustion	:	pUC18 Control Plasmid DNA	No specific data.
		1.42 M 2-Mercaptoethanol	Decomposition products may include the following materials: carbon oxides sulfur oxides
		XL1-Blue Competent Cells	Decomposition products may include the following materials: carbon oxides sulfur oxides halogenated compounds metal oxide/oxides

Extinguishing media

Suitable	:	pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
		1.42 M 2-Mercaptoethanol	Use an extinguishing agent suitable for the surrounding fire.
		XL1-Blue Competent Cells	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

Personal precautions	:	pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		1.42 M 2-Mercaptoethanol	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		XL1-Blue Competent Cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	:	pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		1.42 M 2-Mercaptoethanol	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		XL1-Blue Competent Cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up			
Small spill	:	pUC18 Control Plasmid DNA	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		1.42 M 2-Mercaptoethanol	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		XL1-Blue Competent Cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling	:	pUC18 Control Plasmid DNA	Wash thoroughly after handling.
		1.42 M 2-Mercaptoethanol	Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
		XL1-Blue Competent Cells	Do not ingest. Wash thoroughly after handling.

7 . Handling and storage

Storage : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
1.42 M 2-Mercaptoethanol	
2-Mercaptoethanol	**AIHA WEEL (United States, 1/2008).** TWA: 0.2 ppm 8 hour(s).
XL1-Blue Competent Cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust
Manganese dichloride	**ACGIH TLV (United States, 1/2008).** TWA: 0.2 mg/m³, (as Mn) 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** CEIL: 5 mg/m³, (as Mn) **NIOSH REL (United States, 12/2001).** TWA: 1 mg/m³, (as Mn) 10 hour(s). STEL: 3 mg/m³, (as Mn) 15 minute(s). **OSHA PEL (United States, 11/2006).** CEIL: 5 mg/m³, (as Mn)
Sucrose	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction **NIOSH REL (United States, 12/2001).** TWA: 10 mg/m³ 10 hour(s). Form: Total TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction **OSHA PEL (United States, 11/2006).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
Dimethyl sulfoxide	**AIHA WEEL (United States, 1/2008).** TWA: 250 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

8 . Exposure controls/personal protection

Skin : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Other protection : Not available.

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	: pUC18 Control Plasmid DNA	Liquid.
	1.42 M 2-Mercaptoethanol	Liquid.
	XL1-Blue Competent Cells	Liquid.
Flash point	: pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.
Color	: pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Not available.
Odor	: pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Not available.
pH	: pUC18 Control Plasmid DNA	Neutral.
	1.42 M 2-Mercaptoethanol	Neutral.
	XL1-Blue Competent Cells	Neutral.
Boiling/condensation point	: pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
	1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
	XL1-Blue Competent Cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F)
Melting/freezing point	: pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	1.42 M 2-Mercaptoethanol	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	XL1-Blue Competent Cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 3.02°C (37.4°F)

9 . Physical and chemical properties

Relative density

pUC18 Control Plasmid DNA	Not available.
1.42 M 2-Mercaptoethanol	Only known value: 1.1 (Water = 1) (2-Mercaptoethanol).
XL1-Blue Competent Cells	Weighted average: 1.29 (Water = 1)

Vapor pressure

pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
1.42 M 2-Mercaptoethanol	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C)
XL1-Blue Competent Cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.11 kPa (15.83 mm Hg) (at 20°C)

Vapor density

pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).
1.42 M 2-Mercaptoethanol	Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1)
XL1-Blue Competent Cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.98 (Air = 1)

Evaporation rate

pUC18 Control Plasmid DNA	Not available.
1.42 M 2-Mercaptoethanol	Not available.
XL1-Blue Competent Cells	0.026 (Dimethyl sulfoxide) compared with Butyl acetate.

10 . Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Highly reactive or incompatible with the following materials: oxidizing materials and organic materials.
Reactive or incompatible with the following materials: acids.

Hazardous decomposition products

pUC18 Control Plasmid DNA	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
1.42 M 2-Mercaptoethanol	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
XL1-Blue Competent Cells	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Conditions of reactivity - Flammability : Flammable in the presence of the following materials or conditions: open flames, sparks and static discharge.

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Dimethyl sulfoxide	LD50 Dermal	Rat	40 gm/kg	-
	LD50 Oral	Rat	14500 mg/kg	-
Sucrose	LD50 Oral	Rat	29700 mg/kg	-
Manganese dichloride	LD50 Oral	Rat	250 mg/kg	-
Glycerol	LD50 Dermal	Rabbit	>10 gm/kg	-
	LD50 Oral	Rat	12600 mg/kg	-
Potassium chloride	LD50 Oral	Rat	2600 mg/kg	-

Eyes

pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
1.42 M 2-Mercaptoethanol	Irritating to eyes.
XL1-Blue Competent Cells	No known significant effects or critical hazards.

11 . Toxicological information

Skin	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
		XL1-Blue Competent Cells	Toxic if swallowed.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
XL1-Blue Competent Cells						
Sucrose	A4	-	-	-	-	-

Potential chronic health effects

Chronic effects	:	Contains material that may cause target organ damage, based on animal data.
Carcinogenicity	:	No known significant effects or critical hazards.
Mutagenicity	:	No known significant effects or critical hazards.
Teratogenicity	:	No known significant effects or critical hazards.
Developmental effects	:	No known significant effects or critical hazards.
Fertility effects	:	No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation	:	No specific data.
Ingestion	:	No specific data.
Skin	:	No specific data.
Eyes	:	No specific data.

Target organs	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Other adverse effects	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

12 . Ecological information

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Dimethyl sulfoxide	-	Acute LC50 35 to 37 ml/L Fresh water	Fish	96 hours
	-	Acute LC50 34000000 ug/L Fresh water	Fish	96 hours
Manganese dichloride	-	Acute EC50 4700 ug/L Fresh water	Daphnia	48 hours
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Potassium chloride	-	Acute EC50 83000 ug/L Fresh water	Daphnia	48 hours
	-	Acute LC50 337 mg/L Fresh water	Daphnia	48 hours
	-	Acute LC50 435000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification :

pUC18 Control Plasmid DNA	Not regulated.
1.42 M 2-Mercaptoethanol	Toxic material Irritating material Sensitizing material
XL1-Blue Competent Cells	Toxic material Target organ effects

15 . Regulatory information

	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
U.S. Federal regulations :	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
	1.42 M 2-Mercaptoethanol	**United States inventory (TSCA 8b):** All components are listed or exempted.
	XL1-Blue Competent Cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
	pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
	1.42 M 2-Mercaptoethanol	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: 2-Mercaptoethanol **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard
	XL1-Blue Competent Cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose; Dimethyl sulfoxide **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard; Dimethyl sulfoxide: Immediate (acute) health hazard, Delayed (chronic) health hazard
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 307**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 307**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 311**: Edetic acid
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 311**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 311**: No products were found.

15 . Regulatory information

pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.

SARA 313

	Product name	CAS number	Concentration
Form R - Reporting requirements :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations : pUC18 Control Plasmid DNA

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the

15 . Regulatory information

	components are listed. **Rhode Island Hazardous Substances**: None of the components are listed.
1.42 M 2-Mercaptoethanol	**Connecticut Carcinogen Reporting**: None of the components are listed. **Connecticut Hazardous Material Survey**: None of the components are listed. **Florida substances**: None of the components are listed. **Illinois Chemical Safety Act**: None of the components are listed. **Illinois Toxic Substances Disclosure to Employee Act**: None of the components are listed. **Louisiana Reporting**: None of the components are listed. **Louisiana Spill**: None of the components are listed. **Massachusetts Spill**: None of the components are listed. **Massachusetts Substances**: The following components are listed: 2-Mercaptoethanol **Michigan Critical Material**: None of the components are listed. **Minnesota Hazardous Substances**: None of the components are listed. **New Jersey Hazardous Substances**: None of the components are listed. **New Jersey Spill**: None of the components are listed. **New Jersey Toxic Catastrophe Prevention Act**: None of the components are listed. **New York Acutely Hazardous Substances**: None of the components are listed. **New York Toxic Chemical Release Reporting**: None of the components are listed. **Pennsylvania RTK Hazardous Substances**: The following components are listed: 2-Mercaptoethanol **Rhode Island Hazardous Substances**: None of the components are listed.
XL1-Blue Competent Cells	**Connecticut Carcinogen Reporting**: None of the components are listed. **Connecticut Hazardous Material Survey**: None of the components are listed. **Florida substances**: None of the components are listed. **Illinois Chemical Safety Act**: None of the components are listed. **Illinois Toxic Substances Disclosure to Employee Act**: None of the components are listed. **Louisiana Reporting**: None of the components are listed. **Louisiana Spill**: None of the components are listed. **Massachusetts Spill**: None of the components are listed. **Massachusetts Substances**: The following components are listed: Glycerol;Sucrose **Michigan Critical Material**: None of the components are listed. **Minnesota Hazardous Substances**: None of the components are listed. **New Jersey Hazardous Substances**: The following components are listed: Manganese dichloride **New Jersey Spill**: None of the components are listed. **New Jersey Toxic Catastrophe Prevention Act**: None of the components are listed. **New York Acutely Hazardous Substances**: None of the components are listed. **New York Toxic Chemical Release Reporting**: None of the

15 . Regulatory information

components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride;Sucrose
Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

Label requirements

pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

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Version : 1

Notice to reader

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Indicates information that has changed from previously issued version.

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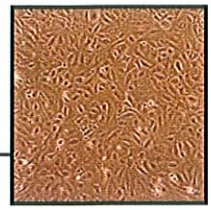
Clonetics® Iliac Artery Endothelial Cell Systems



HIAEC

Introduction

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

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

Cell System Components

- One Iliac Artery Endothelial Cell Product (Cryopreserved or Proliferating)
- One Endothelial Cell Medium (Growth or BulletKit®) - 500 ml
- 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 ml; R3-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 HIAEC includes immunofluorescent staining. Cells stain positive for acetylated LDL uptake and von Willebrand (Factor VIII) antigen and negative for smooth muscle α-actin.

Performance

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

Quality Control

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

Ordering Information

Cryopreserved Cells

CC-2545	HIAEC	≥ 500,000 cells

Proliferating Cells – Flasks and Multiwell Plates

CC-2645	T-25 Flask
CC-0291	T-75 Flask
CC-0095	96-well Plate

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

CC-3202	EGM®-2-MV BulletKit®, EBM®-2 plus SingleQuots® of Growth Supplements	500 ml
CC-3156	EBM®-2, Endothelial Cell Basal Medium-2	500 ml
CC-4147	EGM®-2-MV SingleQuots®, Formulates EBM®-2 to EGM®-2-MV	
CC-5034	ReagentPack™	
	Trypsin Neutralizing Solution	100 ml
	Trypsin/EDTA Solution	100 ml
	HEPES Buffered Saline Solution	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 HIAEC 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.

Lonza

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

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

Related Links

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Cell Micrograph

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Frequently Asked Questions

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Related Cell Culture Products

Protocol:

Subculturing:

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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Cell Biology

ATCC® Number:	CRL-1601™ Order this Item	Price:	$318.00

Designations:	McA-RH7777
Depositors:	JE Becker
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	loosely adherent
Organism:	Rattus norvegicus (rat)
Morphology:	epithelial
Source:	**Organ:** liver **Strain:** Buffalo **Disease:** hepatoma; Morris hepatoma 7777
Cellular Products:	alpha-fetoprotein (AFP, alpha fetoprotein)
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Receptors:	glucocorticoid
Gender:	female
Comments:	Addition of glucocorticoids (dexamethasone) to the medium accelerates cell proliferation and reduces alpha fetoprotein production.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Heavy monolayer sloughs off; subculture before 70% confluency. Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. 1. Remove culture medium with floating cells to a centrifuge tube.2. If any cells are attached, tap flask gently or if necessary add 2.0 to 3.0 ml of 0.25% Trypsin-0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed . 3. Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting. 4.To remove trypsin-EDTA solution, transfer cell suspension to the centrifuge tube with the medium and cells from step #1 and spin at approximately 125 xg for 5 to10 minutes.5. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. 6. Place culture vessels in incubators at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 weekly is recommended **Medium Renewal:** Add medium every 2 to 3 days, do not discard floating cells.
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	recommended serum:ATCC 30-2020 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
References:	26103: . . Recent Results Cancer Res. 44: 103-114, 1974. 32449: Kulas DT, et al. The transmembrane protein-tyrosine phosphatase LAR modulates signaling by multiple receptor tyrosine kinases. J. Biol. Chem. 271: 748-754, 1996. PubMed: 8557682 32463: Schock D, et al. An auxiliary factor containing a 240-kDa protein complex is involved in apolipoprotein B RNA editing. Proc. Natl. Acad. Sci. USA 93: 1097-1102, 1996. PubMed: 8577721 58043: et al., Becker JETwo new rat hepatoma cell lines for studying the unbalanced blocked ontogeny hypothesis*In:* et al., Becker JEOnco-developmental gene expressionNew YorkAcademic Presspp. 259-270, 1976

Related Links

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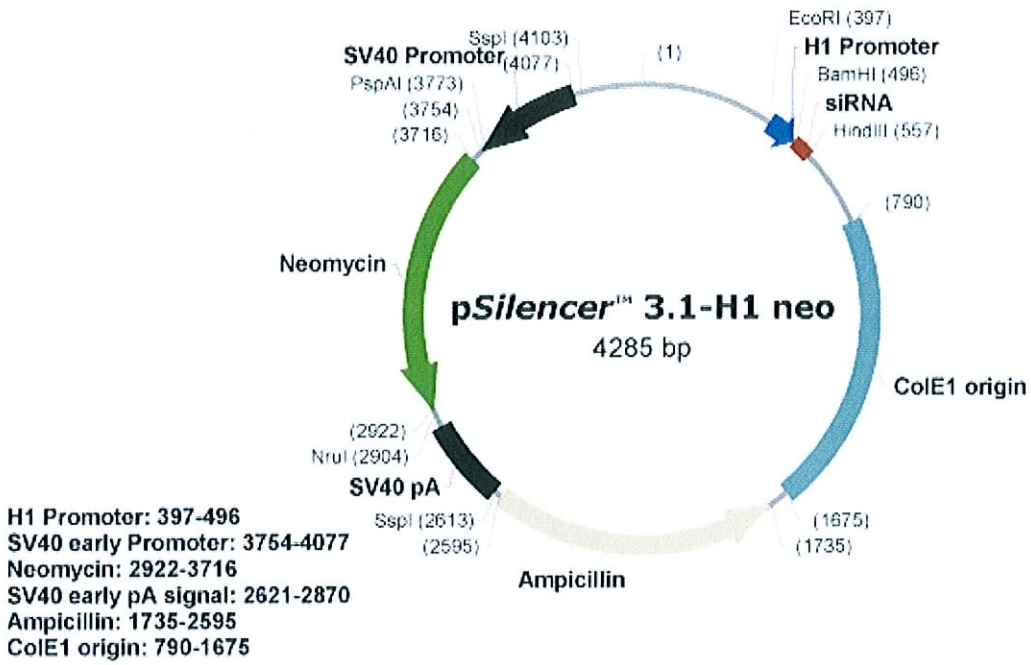
p*Silencer*™ 3.1-H1 neo

Vector Map • Promoter/Cloning Site Region • Restriction Sites • Text-Based Sequence

p*Silencer*™ 3.1-H1 neo Vector Map



H1 Promoter: 397-496
SV40 early Promoter: 3754-4077
Neomycin: 2922-3716
SV40 early pA signal: 2621-2870
Ampicillin: 1735-2595
ColE1 origin: 790-1675

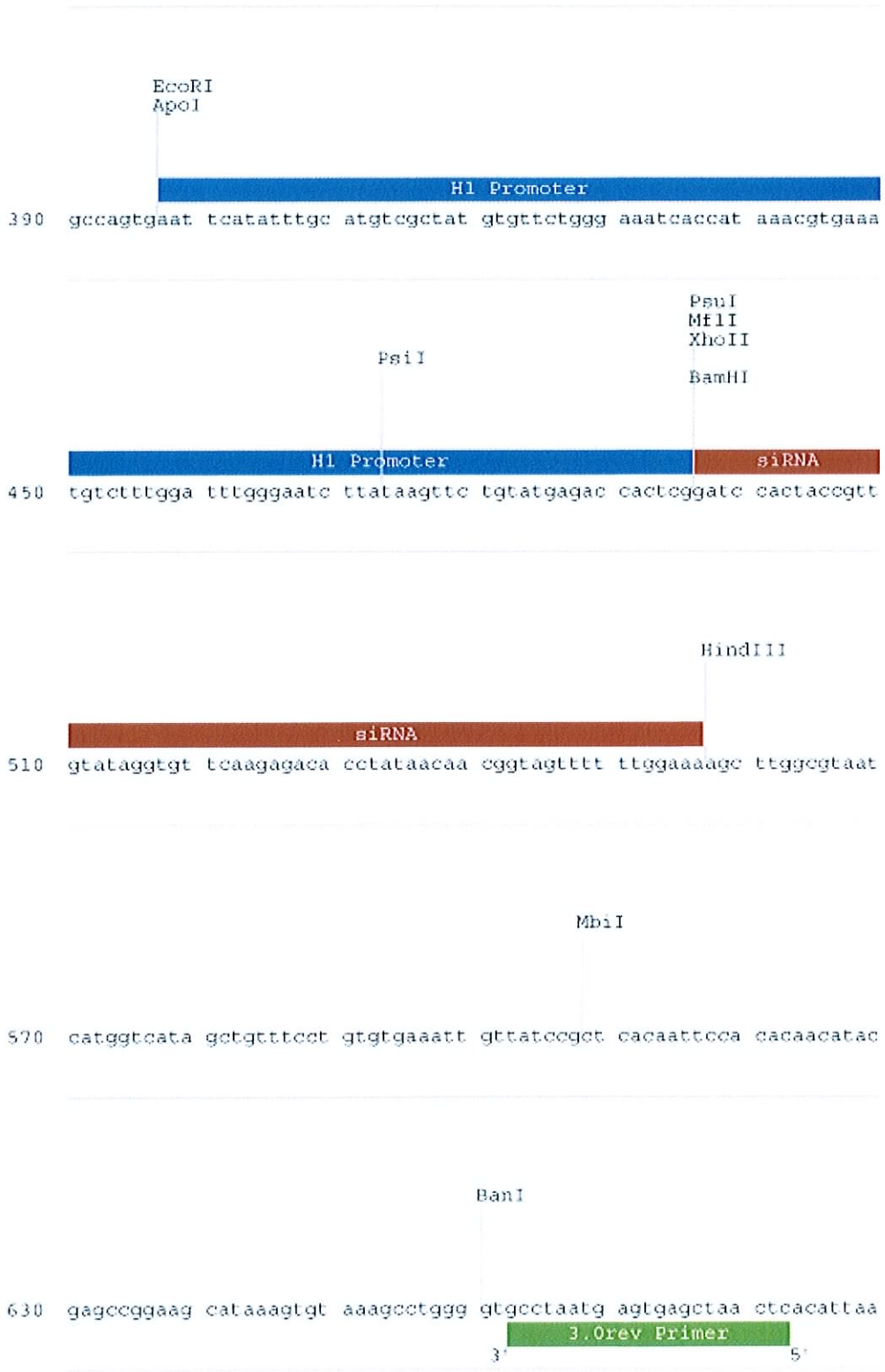
This map and the annotated sequence below portrays the circular negative control vector provided with the kit. It includes the negative control siRNA template (66 base pairs) between the BamH I and Hind III sites.

p*Silencer*™ 3.1-H1 neo Annotated Promoter/Cloning Site Region

We recommend the following primers for sequencing:

M13F(-40): 5'-GTTTTCCCAGTCACGAC-3'

3.0rev: 5'-GAGTTAGCTCACTCATTAGGC-3'


EcoRI
ApoI
H1 Promoter
390 gccagtgaat tcatatttgc atgtcgctat gtgttctggg aaatcaccat aaacgtgaaa
PsuI
MfeI
XhoII
PsiI
BamHI
H1 Promoter
siRNA
450 tgtctttgga tttgggaatc ttataagttc tgtatgagac cactcggatc cactaccgtt
HindIII
siRNA
510 gtataggtgt tcaagagaca cctataacaa cggtagtttt ttggaaaagc ttggcgtaat
MboI
570 catggtcata gctgtttcct gtgtgaaatt gttatccgct cacaattcca cacaacatac
BanI
630 gagccggaag cataaagtgt aaagcctggg gtgcctaatg agtgagctaa ctcacattaa
3.0rev Primer
3'
5'
690 ttgcgttgcg ctcactgccc gctttccagt cgggaaac

p*Silencer*™ 3.1-H1 neo Restriction Sites

Vector Size: 4285 bp

Enzymes with single Restriction Sites

Site	Enzyme
4220	AatII
915	AflIII
1808	AhdI
1331	AlwNI
3772	AvaI
3793	AvrII
495	BamHI
3226	BanII
2313	BcgI
2347	BcgI'
3742	BclI
3283	BsaAI
3728	BsaBI
790	BsaXI
760	BsaXI'
3803	BseRI
45	BsmBI
2667	BsmI
3186	BssHII
3677	EagI
4274	EcoO109I
396	EcoRI
556	HindIII
3505	MscI
184	NdeI
2903	NruI
915	PciI
3538	PstI
3068	RsrII
2288	ScaI
4025	SexAI
3774	SmaI

Non-Cutting Enzymes :

Enzyme
AarI
Acc65I
AccI
AfeI
AflII
AgeI
AleI
AloI
AloI'
ApaI
AscI
AsiSI
BaeI
BaeI'
BbsI
BbvCI
BglII
BlpI
BmgBI
BmtI
Bpu10I
BsgI
BsiWI
BspEI
BsrGI
BstBI
BstEII
BstXI
BstZ17I
Bsu36I
BtrI
ClaI

3798	StuI	DraIII
3468	Tth111I	EcoICRI
3772	XmaI	EcoNI
2407	XmnI	EcoRV
4218	ZraI	FseI
		FspAI
		HincII
		HpaI
		KpnI
		MfeI
		MluI
		NheI
		NotI
		PacI
		PflMI
		PmeI
		PmlI
		PpuMI
		PshAI
		PspOMI
		PsrI
		PsrI'
		SacI
		SacII
		SalI
		SanDI
		SbfI
		SfiI
		SgfI
		SgrAI
		SnaBI
		SpeI
		SrfI
		SwaI
		TaqII'
		XbaI
		XcmI
		XhoI

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.

Also, please include the text "Addgene plasmid 1792" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.