

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:	Savita Dhanvantari
DEPARTMENT:	Medical Biophysics
ADDRESS:	Lawson Health Research Institute
PHONE NUMBER:	65738
EMERGENCY PHONE NUMBER(S):	519-433-2338
EMAIL:	sdhanvan@uwo.ca

Location of experimental work to be carried out :

Building : SJHC	Room(s): F4-127a
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: **Current: UWO, MRI Ontario. Applied for: CIHR, NSERC, Canadian Diabetes Association**

GRANT TITLE(S): **1) Molecular Imaging of Diabetic Cardiomyopathy 2) Mechanisms of Proglucagon Sorting (NSERC)**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Rebecca McGirr	rmcgirr@lawsonimaging.ca	October 2005
Leonardo Guizzetti	lguizzet@uwo.ca	September 2009
Ian Cameron	icamero2@uwo.ca	October 2011
Ahad Al-Hakim	aalhaki@uwo.ca	October 2011

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

Project 1 (NSERC funded)

Background

Cells from organs that secrete hormones are called endocrine cells. As an endocrine cell develops, it changes its shape, regulates its growth, and develops the capacity to secrete hormones. We wish to study the cellular mechanisms of how these cells secrete hormones. We are particularly interested in how endocrine cells in the pancreas secrete a hormone called glucagon, which is important in maintaining blood sugar levels, and is not secreted properly in the diabetic state.

Hypothesis

The secretion of glucagon is controlled by a signal within its structure, and by interactions with other proteins.

Methods

- 1) We shall engineer endocrine cells to produce mutant forms of glucagon and study their secretion patterns. Glucagon will be mutated at sites we think are critical for its secretion. If the mutated forms of glucagon are not secreted properly, we will have uncovered a mechanism for the secretion of glucagon that lies within its structure.
- 2) Glucagon may interact with other proteins to be "chaperoned" to its proper place in the cell for secretion. We shall examine the roles that other known proteins may play in "chaperoning" glucagon and will identify potential new proteins using a technique called proteomics.

Expected Results and Significance

We expect to discover a mechanism for the secretion of glucagon from the pancreas, which will help in identifying what happens in diabetes when glucagon is not secreted properly.

Project 2 (ADF-funded, CIHR and CDA applied for)

Background: Heart disease is a common complication of long-standing diabetes; over 70% of people with diabetes will die due to cardiovascular disease. One such complication is cardiomyopathy, where the heart becomes enlarged and less efficient at pumping blood. Currently, the diagnosis of heart disease is made using imaging technologies such as ultrasound, or magnetic resonance imaging (MRI). These imaging technologies are very good at detecting cardiomyopathy at a late stage. Developing technologies to detect cardiomyopathy at an early stage would enable the development of therapies that slow down the progression of heart disease.

Objectives: Our goal is to develop an imaging technology to detect the early changes in the heart that lead to the cardiomyopathy of diabetes. Our strategy will be use positron emission tomography (PET), a very powerful technology that has the potential to detect changes in heart proteins that act as predictors of heart disease.

Description of research project: PET imaging works by detecting the accumulation of an imaging agent, comprised of a chemical labelled with some radioactivity, in the tissue. We will design the imaging agent to target a specific protein only produced by the heart. We will then test the agents in normal mice to see if it can image the heart tissue, and then in diabetic mice to assess the onset and progression of heart disease. These experiments will show if our imaging agent can detect changes in the heart before the symptoms of chronic diabetic heart disease become evident.

Relevance to diabetes: Our eventual goal is to develop our PET imaging agent into a unique tool in the diagnosis of diabetic heart disease at an early stage, before the heart becomes permanently enlarged. Therefore, our research may lead to developing therapies that can slow the development of cardiomyopathy. This will result in improving the quality of life for people with diabetes, and decrease the burden on the Canadian health care system.

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:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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
<i>JM109</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	2	Fisher	<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>E. coli</i>	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	see attached sheet	1-2	see attached sheet
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid	Invitrogen	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input checked="" type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 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?
JM109	pcDNA3.1	Invitrogen	proglucagon, EGFP, tdTomato, R1a	no	no	none

E. coli

equivalent if available.

for the following strains of E. coli:

[/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 **C57BL/6 mice**

7.3 AUS protocol # **2008-117**

7.4 List the location(s) for the animal experimentation and housing. **SJHC ACF, SJHC F5-104**

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 | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Pound source cats | <input type="checkbox"/> YES | <input type="checkbox"/> NO |
| ◆ Cattle, sheep or goats | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Non-human primates | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Wild caught animals | <input type="checkbox"/> YES, species & colony # | <input type="checkbox"/> NO |
| ◆ Birds | <input type="checkbox"/> YES, species | <input type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify | <input type="checkbox"/> 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 LD₅₀ (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 NO
If NO, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement...
Enter Your Name Savita Dhanvantari **Date:** 05/03/12

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
 YES, location and date of most recent biosafety inspection: **SJHC 2008**
 NO, please certify
 NOT REQUIRED for Level 1 containment

*Mark
26/2012*

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-LHRI-0077**

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:

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 *SD* **Date:** *26-03-12*

15.4 Additional Comments: _____

See E-mail

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): *JAD*
SIGNATURE: _____
Date: *March 26, 2012*

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

Special Conditions of Approval:

Subject: Re: Biological Agents Registry Form: Dhanvantari
From: Savita Dhanvantari <sdhanvan@lawsonimaging.ca>
Date: 4/3/2012 10:15 AM
To: Jennifer Stanley <jstanle2@uwo.ca>

Answers are below.

E-mail

On 29/03/12 4:49 PM, Jennifer Stanley wrote:

Hi there

Thanks for your recent submission. There were a couple of questions that were omitted.
Please send the responses by e-mail:

Section 5.0

HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens NO

Section 15

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? 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:

Wash area with soap and water (or use eyewash station if eyes are exposed) and report to Occupational Health.

Regards,
Jennifer

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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 considered to be level 1 animal pathogens:

- | | | | | |
|---------------|--------------------|-----------|-------------------|----------------|
| • 5K | • CIE85 | • J52 | • MC4100 (MuLac) | • U5/41 |
| • 58 | • DH1 | • J53 | • MG1655 | • W208 |
| • 58-161 | • DH10 GOLD | • JC3272 | • MM294 | • W945 |
| • 679 | • DH10B | • JC7661 | • MS101 | • W1485 |
| • 1532 | • DH5 | • JC9387 | • NC-7 | • W3104 |
| • AB284 | • DH5-alpha | • JF1504 | • Nissle 1917 | • W3110 |
| • AB311 | • DP50 | • JF1508 | • One Shot STBL3 | • WA704 |
| • AB1157 | • DY145 | • JF1509 | • OP50 | • WP2 |
| • AB1206 | • DY380 | • JJ055 | • P678 | • X1854 |
| • AG1 | • E11 | • JM83 | • PA309 | • X2160T |
| • B | • EJ183 | • JM101 | • PK-5 | • X2541 |
| • BB4 | • EL250 | • JM109 | • PMC103 | • X2547T |
| • BD792 | • EMG2 | • K12 | • PR13 | • XL1-BLUE |
| • BL21 | • EPI 300 | • KC8 | • Rri | • XL1-BLUE-MRF |
| • BL21 (DE3) | • EZ10 | • KA802 | • RV308 | • XL0LR |
| • BM25.8 | • FDA Seattle 1946 | • KAM32 | • S17-1λ -PIR | • Y10 |
| • C | • Fusion-Blue | • KAM33 | • SCS1 | • Y1090 (1090) |
| • C-1a | • H1443 | • KAM43 | • SMR10 | • YN2980 |
| • C-3000 | • HF4714 | • LE450 | • SOLR | • W3110 |
| • C25 | • HB101 | • LE451 | • SuperchargeEZ10 | • WG1 |
| • C41 (DE3) | • HS(PFAMP)R | • LE452 | • SURE | • WG439 |
| • C43 (DE3) | • Hfr3000 | • MB408 | • TOP10 | • WG443 |
| • C600 | • Hfr3000 X74 | • MBX1928 | • TG1 | • WG445 |
| • Cavalli Hfr | • HMS174 | • MC1061 | | |

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,

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

Info on Cell Line(s)

Cell lines established in our lab.

1a. CHO-GFP: CHO-K1 cells (obtained from ATCC, information sheet attached) stably transfected with the pEGFP-N1 plasmid.

1b. CHO-GHS-R1a: CHO-K1 cells stably transfected with the GHS-R1a gene (obtained from Missouri S&T cDNA Resource Center) inserted into pcDNA3.1 (1).

1c. CHO-GLP1R-tdtomato: CHO-GLP1R cells (2)(obtained from Dr. Michael Wheeler, University of Toronto) stably transfected with the tdTomato gene (obtained from Dr. John Lewis, LRCP) inserted into pcDNA3.1.

2. PC12/Fc-glucagon: PC12 cells (obtained from Dr. Walter Rushlow, Western University) stably transfected with a fusion gene, consisting of mouse IgG heavy chain fragment, Fc (3) (obtained from Dr. Timothy Reudelhuber, Clinical Research Institute of Montreal) fused in-frame to glucagon (4)(obtained from Dr. Don Steiner, University of Chicago), inserted into pcDNA3.1.

3a. α TC1-6 tkgfp clones 1 (low expresser) and 5 (high expresser)

3b. INS-1 832/13 tkgfp clones 2 (low expresser) and 3 (high expresser)

3c. GLUTag tkgfp

- α TC1-6 cells (5) were obtained from Dr. Ted Friedman, Charles Drew University School of Medicine, Los Angeles CA, USA. INS-1 832/13 cells (6) were obtained from Dr. Christopher Newgard, Duke University School of Medicine, Durham NC, USA.

GLUTag cells (7) were obtained from Dr. Patricia Brubaker, University of Toronto.

- These cell lines have been stably transfected with a plasmid containing the coding sequence of the herpes simplex virus 1 thymidine kinase (HSV1 tk) fused in-frame to green fluorescent protein (gfp) (8). The expression of the gene is under the control of the CMV promoter. The tkgfp plasmid construct was obtained from Dr. Y. Gelovani, MD Anderson Cancer Center, TX USA.

INS-1 832/13-Ferritin:

These cells have been stably transfected with the human ferritin heavy chain fragment inserted into pcDNA3.1, which was obtained from Dr. J. Koropatnick, London Regional Cancer Program. This gene will cause iron accumulation in the cells, for the purposes of imaging the cells by MRI (9).

INS-1 832/13-MagA:

These cells have been stably transfected with the bacterial gene, MagA, inserted into pcDNA3.1. This plasmid was constructed in our lab. This gene will cause iron accumulation in the cells, for the purposes of imaging by MRI (10).

1. **McGirr R, McFarland MS, McTavish J, Luyt LG, Dhanvantari S** 2011 Design and characterization of a fluorescent ghrelin analog for imaging the growth hormone secretagogue receptor 1a. Regul Pept 172:69-76

2. **Xiao Q, Giguere J, Parisien M, Jeng W, St-Pierre SA, Brubaker PL, Wheeler MB** 2001 Biological activities of glucagon-like peptide-1 analogues in vitro and in vivo. *Biochemistry* 40:2860-2869
3. **Methot D, vanKats JP, Lochard N, Tremblay F, Silversides DW, Reudelhuber TL** 2001 Development and application of a biological peptide pump for the study of the in vivo actions of angiotensin peptides. *Am J Hypertens* 14:38S-43S
4. **Dey A, Lipkind GM, Rouille Y, Norrbom C, Stein J, Zhang C, Carroll R, Steiner DF** 2004 Significance of prohormone convertase 2, PC2, mediated initial cleavage at the proglucagon interdomain site, Lys70-Arg71, to generate glucagon. *Endocrinology* 146:713-727
5. **Powers AC, Efrat S, Mojsov S, Spector D, Habener JF, Hanahan D** 1990 Proglucagon processing similar to normal islets in pancreatic alpha-like cell line derived from transgenic mouse tumor. *Diabetes* 39:406-414
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Cell Line Designation: CHO-K1
ATCC[®] Catalog No. CCL-61

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Cricetulus griseus* (hamster, Chinese)

Tissue: ovary

Gender: female

Morphology: epithelial-like

Growth properties: adherent

VirusSuscept: vesicular stomatitis (Indiana); Getah virus

VirusResist: poliovirus 2; modoc virus; Button Willow virus

Reverse Transcriptase: negative

Karyotype: Chromosome Frequency Distribution 50 Cells: 2n = 22. Stemline number is hypodiploid.

Depositors: T.T. Puck

Comments: The CHO-K1 cells were derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T.T. Puck in 1957. The cells require proline in the medium for growth.

Biosafety Level: 1

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

Use Restrictions

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

Handling Procedure for Frozen Cells

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

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

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm² or a 75 cm² culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

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

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with

phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

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 and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
Subcultivation Ratio: 1:4 to 1:8.

6. Incubate cultures at 37°C.

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

Medium Renewal

Once or twice between subculture.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

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

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(additional references are available in the catalog at www.atcc.org)

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

Product code HUMAN SERUM
Product name Human Serum

Company/Undertaking Identification

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

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800-263-6236

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

2. COMPOSITION/INFORMATION ON INGREDIENTS**Hazardous/Non-hazardous Components**

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

3. HAZARDS IDENTIFICATION**Emergency Overview**

The product contains no substances which at their given concentration, are considered to be hazardous to health
This material is derived from a human source and thus is a Potential Biohazard

Form
Liquid

Principle Routes of Exposure/**Potential Health effects**

Eyes	No information available
Skin	No information available

3. HAZARDS IDENTIFICATION

Inhalation No information available
Ingestion No information available

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313

This product is not regulated by SARA.

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

This product does not contain HAPs.

U.S. State Regulations

California Proposition 65

This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:

Non-controlled

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

16. OTHER INFORMATION

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

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End of Safety Data Sheet

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

Product code 350574
Product name PCDNA3.1/ ZEO(+)

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
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Outside of the U.S. ++1-301-431-8585

For research use only

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The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION**Emergency Overview**

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3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/

Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available
Ingestion May be harmful if swallowed.

Specific effects

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

Target Organ Effects

No information available

HMIS

Health	0
Flammability	0
Reactivity	0

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

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Methods for cleaning up Soak up with inert absorbent material.

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Storage Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Protective gloves

Eye protection Safety glasses with side-shields

Skin and body protection Lightweight protective clothing.

Hygiene measures Handle in accordance with good industrial hygiene and safety practice

Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range °C No data available °F No data available

Melting point/range °C No data available °F No data available

Flash point °C No data available °F No data available

Autoignition temperature °C No data available °F No data available

Oxidizing properties No information available

Water solubility No data available

10. STABILITY AND REACTIVITY

Stability Stable.

Materials to avoid No information available

Hazardous decomposition products No information available

Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/

Potential Health effects

Eyes No information available

Skin No information available

Inhalation No information available

Ingestion May be harmful if swallowed.

Specific effects

Carcinogenic effects
Mutagenic effects
Reproductive toxicity
Sensitization

(Long Term Effects)

No information available
No information available
No information available
No information available

Target Organ Effects

No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects

No information available.

Mobility

No information available.

Biodegradation

Inherently biodegradable.

Bioaccumulation

Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name

Not classified as dangerous in the meaning of transport regulations

Hazard Class

No information available

Subsidiary Class

No information available

Packing group

No information available

UN-No

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