THE UNIVERSITY OF WESTERN ONTARIO

BIOLOGICAL AGENTS REGISTRY FORM

Approved Biohazards Subcommittee: October 14, 2010
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).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) 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

PRINCIPAL INVESTIGATOR	Rennian Wang
DEPARTMENT	Physiology & Pharmacology
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Location of experimental work to be carried out: Building(s)
VRL-WC_ Room(s) _ A7-level, Animal Care; 5th floor, A5-116, Wang's lab Bay2

*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:_CDA, CIHR and NSERC____
GRANT TITLE(S):
--Development of Human Fetal Pancreas (CDA);
--Role of c-Kit in mediating islet cell growth and function (CIHR),
--Integrin/ECM in islet cell survival and function (NSERC)

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

Name	UWO E-mail Address	Date of Biosafety Training
Jinming Li	Jli64@uwo.ca	Sept. 19, 2006
Matthew Riopel	mriopel3@uwo.ca	July 15, 2009
Zhi Chao Feng	zfeng5@uwo.ca	June 16, 2009
Jamie Belo	jbelo@uwo.ca	Sept. 15, 2008
Jessica Lynn Dubrick	jdubric2@uwo.ca	Jan. 13, 2010

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

--Human fetal pancreatic tissue stored either at specific locked -80 freezer or a locked cabinet.

--Human PANC-1, rat INS-1 and AR42J, HEK293 cell lines are stored at the liquid nitrogen tank.

--Streptozotocin: Purchased from Sigma and stored at -20 degree in a container with a specific label. To induce STZ-diabetes, mice will be received 75ug/g body weight (i.p) injection. Staff will use standard precautions of reagent usage and wear appropriate protective clothing, gloves and mask. When animals are injected with STZ, lab coat, mask and eye protection will be worn, and care taken with handling of the needle and syringe. Injectable solution is to be made in fume hood with bench coat covering the bench. Bench coat, needles and syringes, tubes for preparing the solution and gloves will be disposed of as a cytotoxic waste placed into the red 10Gal Biomedical waste pail and labeled with cytotoxic stickers as per LHSC waste handling procedures.

--Tamoxifen: Purchased from Sigma and stored at 4 degree in a container with a specific label. To induce conditional beta1 integrin knock-out, mice will be received 1mg/mouse/per for 5 days i.p injection. Injection will be taken at Conventional area (require non sterile micro isolators). Used dirty cages are never to be taken out of the room. Removal from the animal room to the conventional area can only occur on the 6th day. Handling of mice is double gloved (or forceps) by ACVS staff. Staff will use standard precautions of reagent usage and wear appropriate protective clothing, gloves and mask. When animals are injected with Tamoxifen, lab coat, mask and eye protection will be worn, and care taken with handling of the needle and syringe. Bench coat, needles and syringes, tubes for preparing the solution and gloves will be disposed of as a cytotoxic waste placed into the red 10Gal Biomedical waste pail and labeled with cytotoxic stickers as per LHSC waste handling procedures.

Please include a one page research summary or teaching protocol.

We have shown that stem cell factor (SCF)/c-Kit and beta1 integrin/ECM is involved the proliferation, differentiation and function of b-cell in both rodent and human and will continue to determine the underlying mechanisms and subsequent intracellular signaling pathways.

Rationale:
Extracellular matrix (ECM) has a major role to play in tissue morphogenesis and homeostasis, and exerts its influence through the integrin family of receptors. Recent evidence suggests that interactions between integrins and ECM can profoundly impact beta-cell survival and insulin-production. While, c-Kit, a transmembrane receptor tyrosine kinase and its ligand, stem cell factor (SCF) has also been implicated its critical for early stem cell differentiation in haematopoiesis and gametogenesis and important for both rodent and human pancreatic islets development.
During our previous funding period we demonstrated integrin/ECM to be an important variable in determining whether putative endocrine progenitor cells proliferate or differentiate.
Our studies on c-Kit receptor interacted with SCF suggest that c-Kit is not only a marker of beta-cell precursors, but is also critical for beta-cell proliferation, maturation, function and survival. We will further investigate, at the cellular and molecular level, the mechanisms by which the c-Kit receptor interact with SCF and cross-talking with integrin receptors, and its intracellular signaling pathways regulate beta-cell proliferation, differentiation, function and survival.
To study the developing human fetal pancreas, we characterized the transcription factor profiles of

human fetal (8-21wk) pancreas, focusing on FOXO1, SOX9 and RFX61. However, our ability to identify human fetal islet progenitors as well as the signals that regulate islet cell differentiation remains incomplete.

Hypothesis
The c-Kit receptor is critical for beta-cell proliferation, differentiation, metabolic homeostasis and survival through the activation of specific intracellular pathways within islet beta-cells and islet endothelial cells, and through crosstalk with integrin receptors.

Objectives:
1) To characterize the intracellular signaling pathways by which SCF/c-Kit and integrin/ECM regulate the islet cell growth.
2) To determine if increased or down-regulated levels of c-Kit or beta1 integrin in islet-cells influence islet-cell development, function and survival in normal and diabetic models.
3) To examine the interactions of c-Kit and beta1 integrin in islet cell differentiation, survival and function in vitro and in vivo.
4) To identify a specific transcription factor in regulating human islet cell differentiation.

Approach/Research Plan
1) Examine the metabolic changes in the wild-type and mutant mice [C57BL/6J, c-Kit W-v, c-Kit W-v/Fas lpr double mutation mice with C57BL/6J background, and ROSA26-LacZ/B6;129-Itgb1tm1Efu/J mice with Tamoxifen injection]: glucose tolerance (IPGTT) and insulin tolerance (IPITT) tests
2) Generate the diabetic mouse models: High-fat diet (represent to T2D) and Streptozotocin (represent to T1D)
3) Examine the intracellular signaling pathways and function of the islet cells: isolated mice islets and INS-1 cell line, glucose-stimulated insulin secretion (GSIS) and culture.
4) Investigate the functional role of c-Kit and beta-1 integrin in islet cell differentiation of human fetal pancreas and PANC-1 cell line as well as AR42J cell line.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES xO 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? O YES xO 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)* (Be	Is it known to be a human	Is it known to be an animal	Is it known to be a zoonotic	Maximum quantity to be cultured	Source/ Supplier	PHAC or CFIA Containment

specific)	pathogen? YES/NO	pathogen? YES/NO	agent? YES/NO	at one time? (in Litres)		Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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? xO 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	xO Yes O No	Fetal pancreas and duodenum	Human, #10060
Rodent	xO Yes O No	Rat & mouse pancreatic islets	2008-038-04
Non-human primate	O Yes x O No		
Other (specify)	O Yes x 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	xO Yes O No	PANC-1	2	ATCC
Rodent	xO Yes O No	INS-1, AR42J, HEK293	2 2 2	Dr. Savita Dhanvantari Dr. Tianru Jin InVitrogen
Non-human primate	O Yes x O No			
Other (specify)	O Yes xO 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 xO 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? xO YES O 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	Name of Infectious	PHAC or CFIA Containment

		With An Infectious Agent? YES/UNKNOWN	Agent (If applicable)	Level (Select one)
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	LHSC-OB/GY	O Yes Ox Unknown		O 1 XO 2 O 2+ O 3
Human Organs or Tissues (preserved)	LHSC- Pathology	Not Applicable		Not Applicable

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? O YES xO NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below xO 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.

4.4 Will genetic sequences from the following be involved?
- HIV O YES, please specify ____________ xO NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify ________ xO NO
- SV 40 Large T antigen O YES xO NO
- E1A oncogene O YES xO NO
- Known oncogenes O YES, please specify ____________ xO NO
- Other human or animal pathogen and or their toxins O YES, please specify ________ xO NO

4.5 Will virus be replication defective? O YES xO NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES xO 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 xO NO

5.3 How will the biological agent be administered? _______N/A_______________

5.4 Please give the Health Care Facility where the clinical trial will be conducted:___N/A__________

5.5 Has human ethics approval been obtained? xO YES, number: __10600____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used__B6 mice_________________________________

6.3 AUS protocol # ____2008-038-4____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: __________ xO NO

6.5 Will the agent(s) be shed by the animal: O YES xO NO, please justify:

____

____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES xO No If no, please proceed to section 8.0

7.2 Will live animals be used? xO YES O No

7.3 If yes, please specify the animal(s) used:

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

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

____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)___Streptozotocin__and Tamoxifen__________________________
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___STZ 50 to100mg/kg, i.p______________________

8.4 How much of the toxin is handled at one time*? _STZ 75mg/kg, i.p,; Tamoxifen 1mg/mouse________

8.5 How much of the toxin is stored*? ________1g__each ______________________________

8.6 Will any biological toxins be used in live animals? xO YES, Please provide details:_
1. To STZ-induced diabetes: mice will be received STZ 75ug/g body weight with i.p injection by research staff.
2. To induce the conditional beta1 integrin knock-out mice, Tamoxifen Injected i.p. once a day for 5 days at 1mg/mouse by research staff.
________ O NO

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

9.0 Insects

9.1 Do you use insects? O YES xO 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 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
____
____

10.0 Plants

10.1 Do you use plants? O YES xO 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 YES, Please attach the CFIA permit & 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_____ xO NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _________ xO 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE [signature] March 14, 2011

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
xO YES, date of most recent biosafety inspection: MARCH 29, 2011
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-LHRI-0046

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

14.2 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:

For STZ: Staff will use standard precautions of regant usage and wear appropriate protective clothing, gloves and mask. When animals are injected with STZ, lab coat, mask and eye protection will be worn, and care taken with handling of th needle and syringe. Injectable solution is to be made in fum hood with bech coat covering the bench. Bech coat is to be disposed of as a potentically contaminated waste.

For TAMOXIFEN: it may cause abortions in humans, congenital malformation in fetus; eye,skin and respiratory irritation; impair fertility; reproductive disorders. Pregnant women should avoid exposure to this compound Very Toxic, Carcinogen, excreted by mice.
Injection will be taken at Conventional area(require non sterile micro isolators). Used dirty cages are never to be taken out of the room. Removal from the animal room to the conventional area can only occur on the 6th day. Handling of mice is double gloved (or forceps) by ACVS staff.
Dirty bedding scraped clean from cages with a spatula in the BSC, and sent for incineration. Dirty cages should be free (or as free as possible) from bedding for cagewash staff, with dunking out of the room unnecessary as these are chemical hazard s(both)

14.3 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.wph.uwo.ca/

SIGNATURE R. Wang Date: March 21, 2011

15.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Gail Ryder
Date: MARCH 21, 2011

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

Special Conditions of Approval:

Info on Cell Line(s)

Description of Cell Lines and Sources.

INS-1 832/13
Dr. Chris Newgard, Duke University Medical Center

INS-1 cells were originally derived from a rat insulinoma (1). These cells were stably transfected with the human proinsulin gene and maintain expression through 66 population doublings (2). They exhibit glucose-stimulated insulin secretion and are a good model to study beta cell physiology.

αTC1-6
Dr. C. Bruce Verchere, University of British Columbia

αTC1 cells were derived from an SV40 large T antigen transgenic mouse (3). Clonal population 6 showed proglucagon gene expression and no proinsulin or prosomatostatin gene expression. These cells represent a pure population of pancreatic alpha cells and are useful for the study of alpha cell physiology.

InR1-G9
Dr. Patricia Brubaker, University of Toronto.

InR1 cells were derived from a BK-induced hamster glucagonoma. Clone G9 expresses proglucagon very strongly, and processes proglucagon to glucagon, similar to normal alpha cells (4).

GLUTag
Dr. Dan Drucker, The Toronto Hospital and University of Toronto

These cells were derived from a transgenic mouse expressing the SV40 large T antigen in the L cells of the instestine (5). They express proglucagon and process it to the intestinal peptides GLP-1 and GLP-2. These cells are a good model for the study of GLP1-synthesis and secretion (6).

Neuro2A
Dr. Y Peng Loh, National Institutes of Health, Bethesda, MD

Neuro 2A cells were derived from a mouse neuroblastoma. They do not express any prohormone processing enzymes and are ideal for the study of hormone processing and sorting (7).

CHO-GLP1R
Dr. Michael Wheeler, University of Toronto.

CHO cells are derived from Chinese hamster ovary, and these cells have been stably transfected with the human GLP-1 receptor (8). They are used for *in vitro* receptor binding assays.

PC12

Dr. Walter Rushlow, The University of Western Ontario

PC12 cells are originally derived from rat pheochromocytoma. We are using them for the study of hormone processing and sorting.

1. **Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB** 1992 Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 130:167-178
2. **Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB** 2000 Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. Diabetes 49:424-430
3. **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
4. **Drucker DJ, Philippe J, Mojsov S** 1988 Proglucagon gene expression and posttranslational processing in a hamster islet cell line. Endocrinology 123:1861-1867
5. **Drucker DJ, Jin T, Asa SL, Young TA, Brubaker PL** 1994 Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. Mol Endocrinol 8:1646-1655
6. **Brubaker PL, Schloos J, Drucker DJ** 1998 Regulation of glucagon-like peptide-1 synthesis and secretion in the GLUTag enteroendocrine cell line. Endocrinology 139:4108-4114.
7. **Zhang CF, Dhanvantari S, Lou H, Loh YP** 2003 Sorting of carboxypeptidase E to the regulated secretory pathway requires interaction of its transmembrane domain with lipid rafts. Biochem J 369:453-460
8. **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

Isolation of INS-1–Derived Cell Lines With Robust ATP-Sensitive K^+ Channel-Dependent and -Independent Glucose-Stimulated Insulin Secretion

Hans E. Hohmeier, Hindrik Mulder, Guoxun Chen, Rosemarie Henkel-Rieger, Marc Prentki, and Christopher B. Newgard

The biochemical mechanisms involved in regulation of insulin secretion are not completely understood. The rat INS-1 cell line has been used to gain insight in this area because it secretes insulin in response to glucose concentrations in the physiological range. However, the magnitude of the response is far less than that seen in freshly isolated rat islets. In the current study, we have stably transfected INS-1 cells with a plasmid containing the human proinsulin gene. After antibiotic selection and clonal expansion, 67% of the resultant clones were found to be poorly responsive to glucose in terms of insulin secretion (≤2-fold stimulation by 15 mmol/l compared with 3 mmol/l glucose), 17% of the clones were moderately responsive (2- to 5-fold stimulation), and 16% were strongly responsive (5- to 13-fold stimulation). The differences in responsiveness could not be ascribed to differences in insulin content. Detailed analysis of one of the strongly responsive lines (832/13) revealed that its potent response to glucose (average of 10-fold) was stable over 66 population doublings (~7.5 months of tissue culture) with half-maximal stimulation at 6 mmol/l glucose. Furthermore, in the presence of 15 mmol/l glucose, insulin secretion was potentiated significantly by 100 µmol/l isobutylmethylxanthine (320%), 1 mmol/l oleate/palmitate (77%), and 50 nmol/l glucagon-like peptide 1 (60%), whereas carbachol had no effect. Glucose-stimulated insulin secretion was also potentiated by the sulfonylurea tolbutamide (threefold at 3 mmol/l glucose and 50% at 15 mmol/l glucose) and was abolished by diazoxide, which demonstrates the operation of the ATP-sensitive K^+ channel (K_{ATP}) in 832/13 cells. Moreover, when the K_{ATP} channel was bypassed by incubation of cells in depolarizing K^+ (35 mmol/l), insulin secretion was more effectively stimulated by glucose in 832/13 cells than in parental INS-1 cells, which demonstrates the presence of a K_{ATP} channel-independent pathway of glucose sensing. We conclude that clonal selection of INS-1 cells allows isolation of cell lines that exhibit markedly enhanced and stable responsiveness to glucose and several of its known potentiators. These lines may be attractive new vehicles for studies of β-cell function. *Diabetes* 49:424–430, 2000

From BetaGene, Inc. (H.E.H., R.H.-R.); the Departments of Biochemistry and Internal Medicine and the Gifford Laboratories for Diabetes Research (H.M., G.C., C.B.N.), University of Texas Southwestern Medical Center, Dallas, Texas; and the Molecular Nutrition Unit (M.P.), Department of Nutrition, University of Montreal, Centre de Recherche du CHUM, Institut du Cancer, Montreal, Quebec, Canada.

Address correspondence and reprint requests to Christopher B. Newgard, PhD, Gifford Laboratories for Diabetes Research, Room Y8.212, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235. E-mail: newgard@utsw.swmed.edu.

H.E.H. and H.M. contributed equally to this work.

H.E.H. and R.H.-R. are employed by and C.B.N., H.E.H., and R.H-R. hold stock in BetaGene, Inc. C.B.N. is also the founding scientist and a member of the company's board of directors.

Received for publication 27 August 1999 and accepted in revised form 10 November 1999.

GLP-1, glucagon-like peptide 1; HBSS, HEPES balanced salt solution; HPLC, high-performance liquid chromatography; IBMX, isobutylmethylxanthine; K_{ATP}, ATP-sensitive K^+ channel; PD, population doubling; RIA, radioimmunoassay.

The biochemical mechanisms involved in fuel-stimulated insulin secretion are not completely understood (1–3). One impediment to gaining full understanding in this area has been the procurement of insulinoma cell lines that faithfully and stably mimic the performance of β-cells within the normal pancreatic islets of Langerhans. Numerous rodent β-cell lines exhibiting different degrees of differentiation have been reported (3,4). These range from the poorly differentiated RINm5F cell line, which has a low insulin content and no glucose-stimulated insulin secretion (5), to rat cell lines such as INS-1 and mouse cell lines such as MIN-6, βTC6-F7, and βHC9, which have an insulin content closer to that of normal islets and retain some glucose-stimulated insulin secretion (6–10). However, even the best rodent cell lines are imperfect. For example, INS-1 cells generally exhibit only a 2- to 4-fold increase in insulin secretion in response to glucose (6,11,12), which is far less than the 15-fold responses achievable with freshly isolated primary islets (13). Also, MIN-6 cells exhibit secretory responses to pyruvate, which is not a secretagogue for normal islets (14), and βHC9 cells grow very slowly and are thus difficult to study. Finally, loss of differentiated features as a function of time in tissue culture has been reported for several rodent cell lines, including RIN1046-38 and βTC6 (8,15,16). Genetic engineering of RIN1046-38 cells results in clones with stable glucose responsiveness but with maximal insulin secretion occurring at subphysiological glucose concentrations because of a high level of low K_m hexokinase activity in these cells (16–18). Stable glucose responsiveness has also been reported for βTC cells after clonal selection in soft agar (8), but even these cloned cell lines (e.g., βTC6-F7) appear to lose glucose responsiveness after prolonged tissue culture (19).

We have worked extensively with INS-1 cells as a model for metabolic signaling mechanisms in the β-cell (11,12,20,21) and have found that fresh aliquots of these cells at relatively low passage numbers exhibit two- to fourfold responses to glucose over the physiological range, as reported in the study by Asfari et al. (6) that described their isolation. However, further growth of these cells in culture for 2–3 months results in a reduction of the glucose response to twofold or less. INS-1 cells were originally isolated by dispersion of a transplantable radiation-induced INS tumor from NEDH rats into a tissue culture medium containing β-mercaptoethanol (6). Based on the description of methods used in this process (6), INS-1 cells may not be clonal and may instead represent a mixture of endocrine cells with distinct phenotypes. If this is correct, then the loss of differentiated function that we have experienced with INS-1 cells may be explained by preferential expansion of a glucose-unresponsive subpopulation of cells from an initial polyclonal mixture.

To test this possibility, we used a stable transfection strategy to isolate a large number of discrete INS-1–derived colonies. Analysis of the resultant clones shows that the original INS-1 cell line is clonally heterogeneous but also that cell lines with robust secretory responses to glucose can be derived from stable transfection of the original population. These new lines may serve as improved models for studies of β-cell function.

RESEARCH DESIGN AND METHODS

Reagents. All reagents were from Sigma (St. Louis, MO) unless otherwise noted and were used at the concentrations shown in the legends to the figures.

Cell culture. Parental INS-1 cells (6) were a generous gift from Dr. Claes Wollheim (Geneva). These cells and new cell lines derived from them by stable transfection were grown in 10-cm tissue culture dishes at 37°C and 5% CO_2 in a humidified atmosphere. The cells were passaged every 5 days by using 1 ml 0.05% trypsin-EDTA. The culture medium was RPMI-1640 with 11.1 mmol/l D-glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mmol/l HEPES, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, and 50 µmol/l β-mercaptoethanol.

Isolation of new INS-1–derived cell lines with a stable transfection approach. To investigate clonal heterogeneity in INS-1 cells while increasing insulin content, the parental cells were stably transfected with the plasmid pCMV8/INS/IRES/Neo containing the human insulin cDNA as previously described (17). Two independent transfection experiments were performed, each yielding 50–60 discrete colonies that appeared under selection with G418. These colonies were isolated and expanded for further analysis.

Secretion assays. Parental INS-1 cells or cell lines derived from them by using stable transfection were used in these studies. In studies involving the new cell line 832/13, population doublings (PDs) 26–92 were used. The cells were plated onto 24-well plates at a density of ~0.5 × 10^6 cells/well and were grown to 100% confluence before assay. At 18 h before secretion experiments, the standard tissue culture medium containing 11.1 mmol/l glucose was switched to fresh medium containing 5 mmol/l glucose. Insulin secretion was assayed in HEPES balanced salt solution (HBSS) (114 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.16 mmol/l $MgSO_4$, 20 mmol/l HEPES, 2.5 mmol/l $CaCl_2$, 25.5 mmol/l $NaHCO_3$, and 0.2% bovine serum albumin [essentially fatty acid free], pH 7.2). Cells were washed in 1 ml HBSS with 3 mmol/l glucose followed by a 2-h preincubation in 2 ml of the same buffer. Insulin secretion was then measured by using static incubation for a 2-h period in 0.8 ml of HBSS containing the glucose concentrations and/or secretagogues indicated in the figure legends. For studies of K_{ATP} channel-independent insulin secretion (22,23), assays were performed as described above except that 35 mmol/l KCl (depolarizing K^+) was included; consequently, the Na^+ concentration was reduced from 120 to 89.8 mmol/l to maintain osmolarity.

Insulin radioimmunoassay. For determination of total insulin content and for measurement of absolute output of insulin during glucose stimulation of INS-1 lines, samples were analyzed by radioimmunoassay (RIA) with the ImmunoChem Coated Tube Insulin RIA kit (ICN Pharmaceuticals, Costa Mesa, CA). According to the manufacturer, this assay detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. In all other secretion experiments in which data are expressed as fold increases, the Coat-a-Count kit (Diagnostic Products, Los Angeles, CA) was used as previously described (17,18). This assay uses antibodies to human insulin that cross-react ~20% with rat insulin. Human insulin (referenced to U.S. Pharmacopia, Rockville, MD, insulin lot G) was used for standard curves in both assays.

Insulin content and proinsulin processing. To measure cellular insulin content, cell pellets were sonicated in 1 mol/l acetic acid containing 0.1% bovine serum albumin. Aliquots of acid extracts were subjected to RIA with an assay that detects both rat and human insulin (ICN kit) as described above. The extent of proinsulin processing was evaluated by preparing acetic acid extracts of whole cells or culture medium and by analyzing these samples with high-performance liquid chromatography (HPLC) as previously described (17,24).

RESULTS

Clonal heterogeneity of INS-1 cells revealed by stable transfection with the human insulin gene and isolation of individual clones. Parental INS-1 cells were transfected with a plasmid containing the human insulin gene under control of the cytomegalovirus promoter and a neomycin resistance gene. A total of 58 independent colonies were isolated after selection with G418. Colonies in G418-containing medium were discrete and well separated and likely represented populations derived from individual clones. All 58 cell lines were screened by measuring the fold increase in insulin secretion at 15 vs. 3 mmol/l glucose. As shown in Fig. 1, 67% of the clones (39 clones) were poorly responsive to glucose (≤2-fold stimulation by high glucose), 17% of the clones (10 clones) were moderately responsive (2- to 5-fold stimulation), and 16% (9 clones) were strongly responsive (5- to 13-fold stimulation). These results are representative of two independent transfection experiments and indicate that parental INS-1 cells consist of a mixture of cells with different glucose-sensing capacity.

To validate the results obtained in the screen shown in Fig. 1, glucose-stimulated insulin secretion was reevaluated in four "poorly responsive" clones (lines 832/1, 832/2, 832/7, and 832/23) and in four "strongly responsive" clones (lines 832/3, 832/13, 832/21, and 832/24). Insulin secretion was measured with an RIA that detects rat and human insulin equally. The data in Fig. 2 confirm the distinction between the two groups of clones in that the poorly responsive lines were similar to or less responsive than the parental cells, which exhibited a threefold increase in insulin secretion as glucose was increased from 3 to 15 mmol/l. In contrast, all four of the strongly responsive clones exhibited better respon-

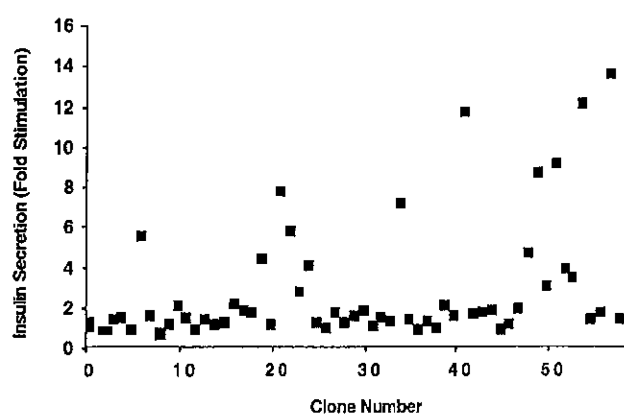

FIG. 1. Screen for glucose-responsive INS-1–derived clones. Parental INS-1 cells were stably transfected with a plasmid containing the human proinsulin gene as described in RESEARCH DESIGN AND METHODS. After antibiotic selection, individual colonies were isolated, expanded, and screened by measuring the fold increase in insulin secretion at 15 vs. 3 mmol/l glucose. Data for 58 individual clones are presented and represent the means of three independent measurements per clone.

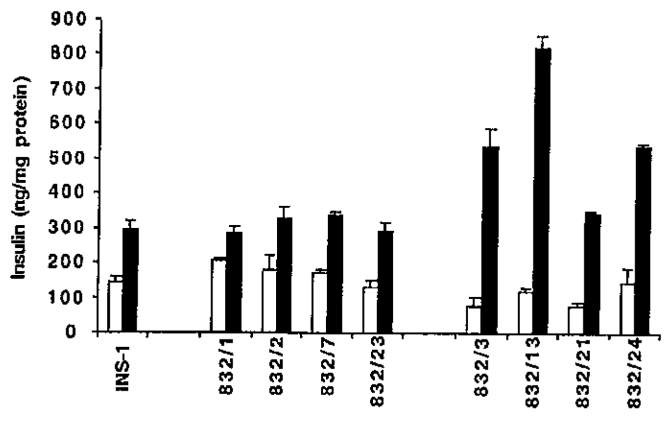

FIG. 2. Glucose-stimulated insulin secretion in INS-1–derived clones. Based on the screen shown in Fig. 1, four clones with poor glucose responsiveness (832/1, 832/2, 832/7, and 832/23) and four strongly responsive clones (832/3, 832/13, 832/21, and 832/24) were selected for further analysis. Insulin secretion was measured at 3 (□) and 15 (■) mmol/l glucose with an RIA that detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. Data represent the means ± SE for three independent experiments, each of which was performed in triplicate.

siveness than parental INS-1 cells (range of 4.4- to 8-fold responses to glucose). Among the highly responsive clones, line 832/13 secreted the largest amount of insulin during glucose stimulation (800 ng · mg^{-1} protein · h^{-1}) and was chosen for further study.

Insulin content of selected INS-1 cell lines. We next investigated whether the superior performance of the strongly responsive group could be related to insulin content. To evaluate this possibility, we measured total insulin content (rat + human insulin) in the clones described in Fig. 2. As shown in Table 1, insulin content was generally increased in the two groups of clones derived from stable transfection of the human insulin gene compared with parental INS-1 cells, but no consistent difference between the two groups of transfected cells was evident. In particular, the insulin content of clone 832/13, the strongly responsive line that was chosen for further study, was 1.5 μg/10^6 cells, which is similar to the content of the four poorly responsive clones (range 1.3–2.3 μg/10^6 cells).

TABLE 1
Insulin content of INS-1–derived cell lines

Response	Cell line	Insulin content (ng/10^6 cells)
2- to 4-fold	Parental INS-1	866 ± 108
Poor (≤2-fold)	832/1	1,265 ± 332
	832/2	1,322 ± 308
	832/7	2,183 ± 254
	832/23	1,722 ± 246
Strong (5- to 13-fold)	832/2	1,165 ± 281
	832/13	1,440 ± 348
	832/21	824 ± 96
	832/24	1,405 ± 111

Data are means ± SE for four independent determinations. Insulin content was measured using an RIA that detects both human and rat insulins with a relative reactivity toward rat insulin of 90% compared with human insulin. Note that, although all but one of the transfected clones (832 series) contained more insulin than the parental INS-1 cells, no consistent difference was evident in content between poorly (first group of four lines) and strongly (second group of four lines) responsive cells.

Stable glucose response and proinsulin processing in a clonal INS-1 cell line. To determine whether clone 832/13 was capable of maintaining potent glucose responsiveness in a stable fashion, insulin secretion assays were performed at 26, 64, and 92 PDs, which represent a total interval of ~7.5 months of tissue culture. As shown in Fig. 3, insulin secretion was stimulated 8-fold at 15 mmol/l glucose relative to secretion at 3 mmol/l glucose in cells at PD 26, 11-fold at PD 64, and 10-fold at PD 92. These results indicate that glucose responsiveness of the selected INS-1 cell line is stably maintained.

We also studied proinsulin processing by using HPLC analysis. As shown in Fig. 4A, parental INS-1 cells contained two prominent absorbance peaks. One comigrated with rat insulin I (17), and the other likely represents rat insulin II based on previous studies of INS-1 cells by Asfari et al. (6) and because insulin was measured in both peaks by RIA. Proinsulin was present at very low levels in the parental INS-1 cells, which suggests that efficient processing of the endogenous rat proinsulin occurred in our starting population (Fig. 4A). The 832/13 cells at PD 26 retained the rat insulin I and II peaks and also exhibited a smaller peak comigrating with a human insulin standard, which confirms expression of the stably introduced human insulin transgene (Fig. 4B). Again, no significant accumulation of proinsulin was evident despite overexpression of the human proinsulin gene. Importantly, human insulin expression and the efficiency of proinsulin processing were identical in 832/13 cells at PD 42 to those at PD 26 (Fig. 4B and C). This provides further evidence of a stable phenotype in cell populations selected by stable transfection.

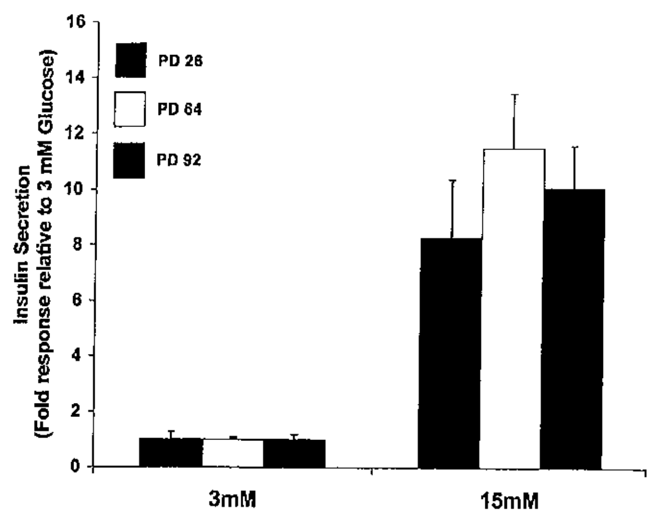

FIG. 3. Stable phenotype of INS-1–derived clone 832/13. Insulin secretion in response to 3 and 15 mmol/l glucose was measured in clone 832/13 at PD 26, PD 64, and PD 92, which indicate a total time interval of ~7.5 months of tissue culture. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE of two independent experiments, each of which was performed in triplicate.

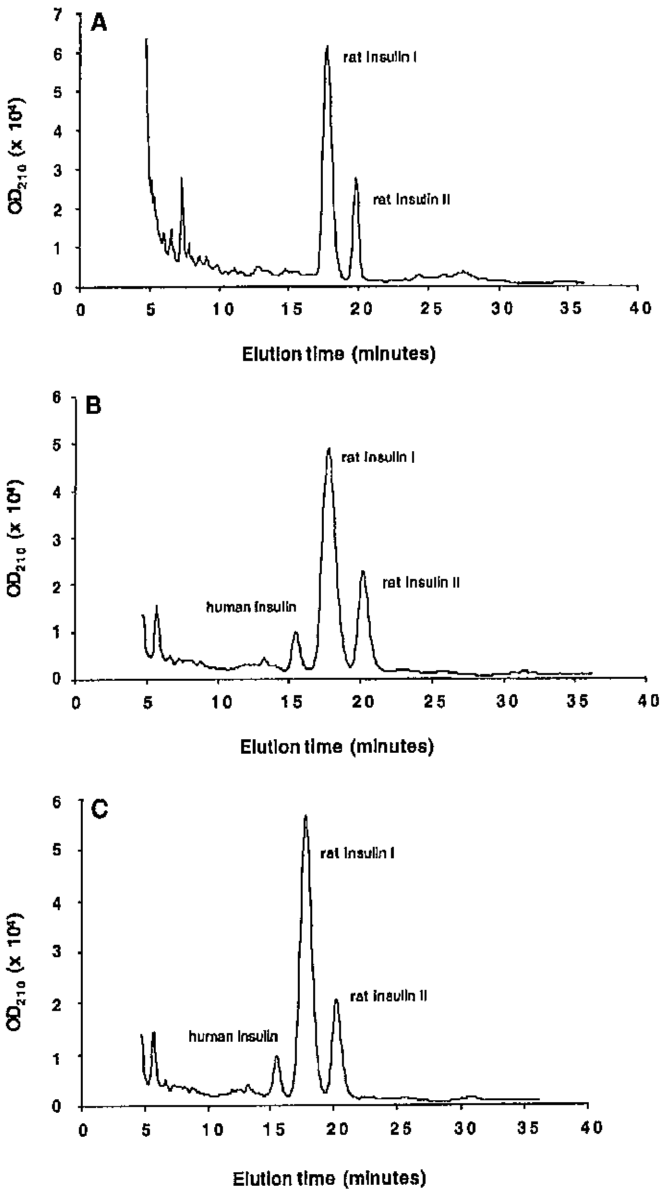

FIG. 4. HPLC analysis of proinsulin processing in INS-1-derived clones. Extracts were prepared as described in RESEARCH DESIGN AND METHODS from parental INS-1 cells (A), clone 832/13 at PD 26 (B), and clone 832/13 at PD 42 (C) and were subjected to HPLC analysis. Parental INS-1 cells contain two prominent absorption peaks, the larger one comigrating with rat insulin I and the other likely representing rat insulin II based on the RIA of fractions from this peak. In clone 832/13 at either PD 26 or 42, the rat insulin peaks remain, and a new peak that comigrates with human insulin appears. Importantly, these fractions contain very little proinsulin, and the HPLC profile is unaltered in 832/13 cells at PD 42 compared with PD 26, which provides further evidence of the phenotypic stability of these cells.

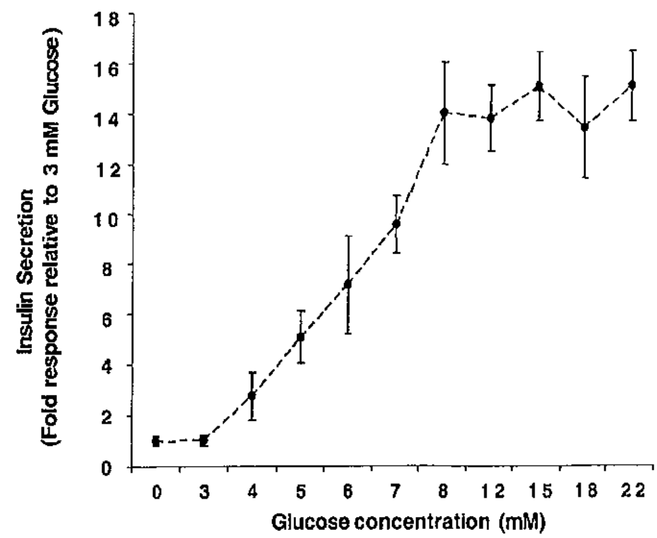

FIG. 5. Glucose dose-response curve for INS-1-derived clone 832/13. The 832/13 cells were incubated at the indicated concentrations of glucose for 2 h. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for five independent experiments.

Glucose dose response in the 832/13 INS-1 cell line. We next evaluated insulin secretion in response to a range of glucose concentrations in 832/13 cells. As shown in Fig. 5, insulin secretion was unchanged as glucose was increased from 0 to 3 mmol/l glucose. Insulin secretion was doubled at 4 mmol/l glucose relative to secretion at 0 or 3 mmol/l and then continued to increase as the glucose concentration increased until a maximal response of 13-fold above baseline was attained at 8 mmol/l glucose. Further increases in glucose caused no additional enhancement in insulin secretion. The magnitude of the response to glucose exhibited by 832/13 cells compares favorably with the responses reported for freshly isolated rat islets (13). However, the glucose dose response was slightly shifted to the left compared with rat islets, which have a threshold for glucose response of 5.5 mmol/l and maximal responses that occur at 16–20 mmol/l glucose (2,3).

Effect of glucose potentiators on the 832/13 INS-1 cell line. Glucose regulates insulin secretion from normal pancreatic islets in concert with numerous physiologically relevant potentiators (1–3). We therefore evaluated the capacity of the 832/13 cell line to respond to such agents. Consistent with the data in Figs. 4 and 5, insulin secretion was stimulated ninefold as glucose was increased from 3 to 15 mmol/l (Fig. 6). Relative to the amount of insulin secreted at 15 mmol/l glucose, further increases of 320, 77, and 60% were achieved by the inclusion of 100 μmol/l of isobutylmethylxanthine (IBMX), 1 mmol/l oleate/palmitate (2:1 molar ratio), or 50 nmol/l glucagon-like peptide 1 (GLP-1), respectively. In contrast to the clear effects of these agents, glucose-stimulated insulin secretion was not potentiated by the inclusion of a muscarinic receptor agonist (100 μmol/l carbachol) (Fig. 6). The lack of effect of carbachol on INS-1 cells is consistent with our previous studies and contrasts with other rat cell lines such as RIN1046-38 and its derivatives, which exhibit a potent carbachol response (21).

K_{ATP} channel-dependent and -independent pathways of insulin secretion in the 832/13 INS-1 cell line. We next investigated the involvement of the K_{ATP} channel in the glucose response of line 832/13 by performing experiments in the presence of a sulfonylurea (tolbutamide), which interacts with the sulfonylurea receptor to cause channel closure, and diazoxide, an opener of the channel. As shown in Fig. 7, treatment of 832/13 cells with 250 μmol/l diazoxide completely blocked the stimulation of insulin secretion caused by

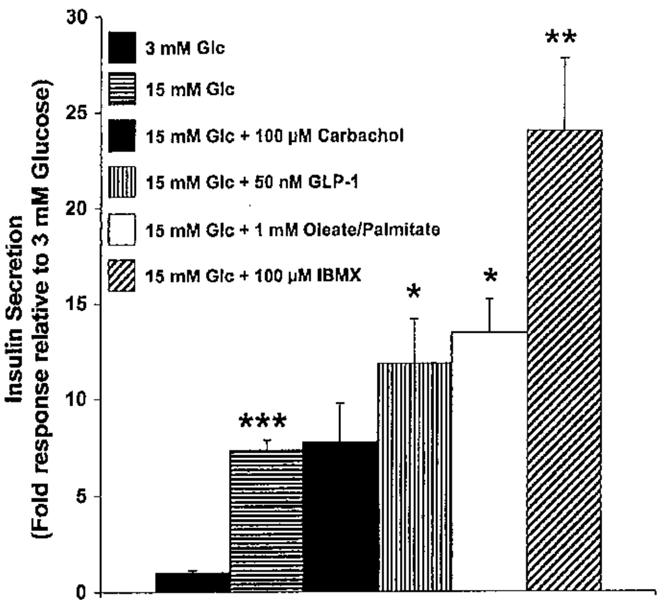

FIG. 6. Potentiation of glucose-stimulated insulin secretion in the INS-1-derived clone 832/13. The 832/13 cells were incubated in low (3 mmol/l) or high (15 mmol/l) glucose (Glc) in the presence and absence of known potentiators of insulin secretion as indicated in the legend. Data represent the means ± SE for five independent experiments. Paired two-tailed analyses were performed by comparing the 15 and 3 mmol/l glucose groups or by comparing 15 mmol/l glucose + potentiator groups with 15 mmol/l glucose alone. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

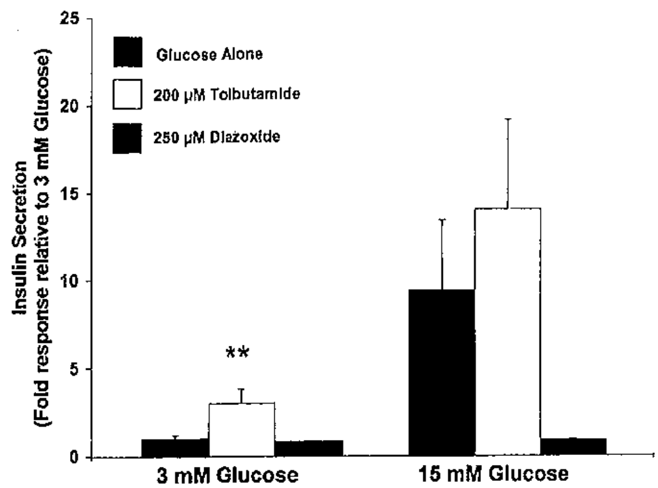

FIG. 7. Evidence for operation of the K_{ATP} channel system in the INS-1-derived clone 832/13. The 832/13 cells were incubated at 3 or 15 mmol/l glucose in the presence or absence of 200 μmol/l tolbutamide or 250 μmol/l diazoxide. Data represent the means ± SE for six independent experiments. **Insulin secretion in the 3 mmol/l glucose + tolbutamide-treated cells was greater than in cells treated with 3 mmol/l glucose alone ($P < 0.01$).

15 mmol/l glucose. In contrast, 200 μmol/l tolbutamide stimulated insulin secretion by threefold at 3 mmol/l glucose and by 50% at 15 mmol/l glucose, although the effect at high glucose did not quite reach statistical significance. The lesser effect of tolbutamide at high glucose is consistent with the very effective action of glucose alone on K_{ATP} channel closure.

In addition to the K_{ATP}-channel-dependent pathway, glucose has been shown to regulate insulin secretion from normal islets in which the K_{ATP} channel was bypassed by treatment with depolarizing K^+ (22,23). We investigated whether this K_{ATP} channel-independent pathway was operative in our new cell lines.

Figure 8 shows insulin secretion from parental INS-1 cells, a representative "poorly responsive" transfected INS-1 clone (834/105), and the "strongly responsive" 832/13 cell line in the presence of 35 mmol/l K^+. This high concentration of K^+ depolarizes the plasma membrane directly, circumventing the requirement for glucose-induced closure of K_{ATP} channels. Under these conditions, both the parental INS-1 cells and the poorly responsive clone exhibited less than a doubling of insulin secretion as glucose was increased from 3 to 15 mmol/l, and opening the K_{ATP} channels with diazoxide did not affect either the basal or stimulated insulin output as expected in the presence of depolarizing K^+. Compared with either of these populations, 832/13 cells exhibited much better K_{ATP} channel-independent signaling in that 15 mmol/l glucose stimulated insulin secretion by 4.5-fold relative to output at 3 mmol/l glucose; diazoxide was again without effect. We also studied the glucose concentration dependence for stimulation of insulin secretion from 832/13 cells by the K_{ATP} channel-independent pathway (in the presence of 35 mmol/l K^+ and diazoxide). We found that the concentration dependence was virtually identical in cells studied at 35 mmol/l K^+ (Fig. 9) compared with cells studied at 4.8 mmol/l K^+ (Fig. 5), although the fold stimulation of insulin secretion was less in the former than in the latter experiments. These data in combination with the experiments in Fig. 7 provide clear evidence of the presence of both K_{ATP} channel-dependent and -independent mechanisms of glucose-stimulated insulin secretion in 832/13 cells.

DISCUSSION

Dual motivations exist for developing INS cell lines that faithfully mimic the function of normal pancreatic islet β-cells. First, our understanding of the precise biochemical mechanisms of fuel-stimulated insulin secretion has developed relatively slowly because of the difficulty inherent in isolating fully functional pancreatic islets from humans or animals. In addition, secretory responses to glucose and its potentiators begin to wane within hours of islet isolation and maintenance in tissue culture. Thus, a cell line that stably responds to physiologically relevant secretagogues could be valuable for defining mechanisms involved in the regulation of insulin secretion, which may possibly lead to the development of new pharmaceutical reagents for treating diabetes. Second, cell lines that faithfully mimic the function of normal pancreatic islets could serve as surrogates for islets in transplantation therapy for type 1 diabetes (25,26). To fulfill this role, candidate cell lines must secrete insulin over the physiological range of glucose concentrations and should also be responsive to known potentiators of glucose signaling. Most importantly, these responses must be stably maintained in both in vitro and in vivo settings. In the present study, we demonstrate that the commonly used INS-1 cell line consists of a mixture of glucose-responsive and -unresponsive cells. We further show that stable transfection of parental INS-1 cells and isolation of discrete colonies result in new cell lines with markedly improved function compared with the original pop-

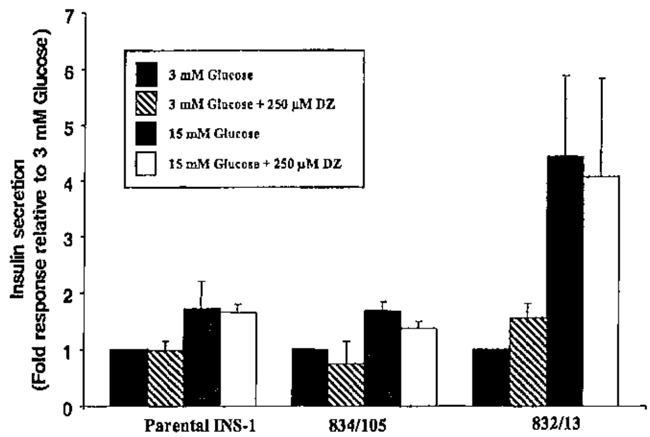

FIG. 8. Improved K_{ATP} channel-independent pathway of glucose-stimulated insulin secretion in INS-1-derived clones. Insulin secretion from parental INS-1 cells, a representative clone poorly responsive to glucose (834/105), and a representative clone strongly responsive to glucose (832/13) was measured in the presence of a depolarizing concentration of K^+ (35 mmol/l), which provides a measure of K_{ATP} channel-independent glucose signaling. Secretion was measured in cells exposed to low (3 mmol/l) or high (15 mmol/l) glucose in the presence and absence of 250 µmol/l diazoxide (DZ) as indicated in the legend. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for three independent experiments.

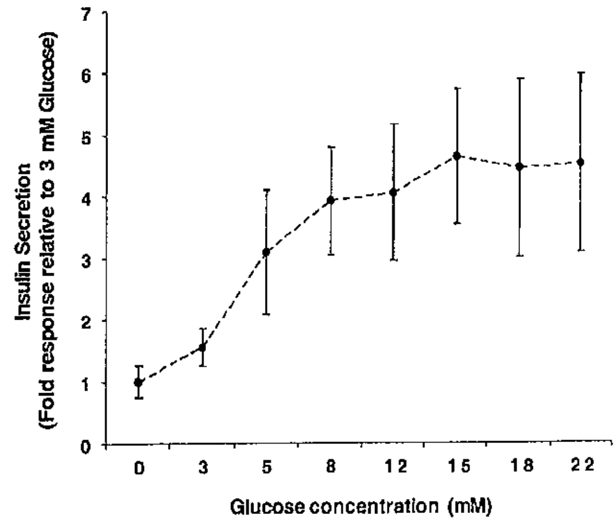

FIG. 9. Glucose dose-response curve for K_{ATP} channel-independent secretion from INS-1-derived clone 832/13. The 832/13 cells were incubated at the indicated concentrations of glucose for 2 h in the presence of 35 mmol/l K^+ and 250 µmol/l diazoxide to allow measurement of K_{ATP} channel-independent signaling. Insulin secreted into the medium was measured with RIA, was normalized to total cellular protein, and was expressed as fold increase relative to insulin secreted at 3 mmol/l glucose. Data represent the means ± SE for four independent experiments.

ulation. The enhanced secretory responsiveness to glucose is maintained for at least 7.5 months in tissue culture, which suggests that the newly derived lines may be a useful new tool for studying the mechanisms of insulin secretion and preclinical transplantation studies in animal models of diabetes.

The apparent clonal heterogeneity of the original INS-1 cell line is understandable in the light of the methods used for its isolation. Asfari et al. (6) dispersed cells from transplantable X-ray–induced INS tumors from NEDH rats and then separated viable cells from aggregates and debris by Percoll gradient centrifugation. The resultant preparation of cells was cocultured with lymphocytes in the presence of β-mercaptoethanol, which was added to enhance survival and proliferation of lymphocytic cells. INS-1 cells were isolated as free-floating cell aggregates from these coculture experiments. Based on this description of methods, such aggregates may have contained cells of different origin and degree of differentiation.

In addition to robust glucose responsiveness, the new INS-1 cell lines isolated with stable transfection exhibit other attractive features. First, the glucose effect is potentiated effectively by agents known to enhance glucose-stimulated insulin secretion from normal pancreatic islets such as IBMX, GLP-1, free fatty acids (a 2:1 oleate/palmitate mixture), and the sulfonylurea tolbutamide. Second, in the presence of physiological K^+ concentrations, the new lines exhibit a complete inhibition of insulin secretion by the K_{ATP} channel opener diazoxide, which indicates that regulation of this channel is an important component of their glucose response, as is the case in normal islets. Third, the new lines have a much more pronounced K_{ATP} channel-independent glucose-sensing pathway than parental INS-1 cells such that the new cells have responses similar to those noted in normal islets by Gembal et al. (22). These findings suggest that the new lines will be improved models for studying key issues in β-cell stimulus/secretion coupling. For example, our recent study showing a lack of effect of a malonyl CoA decarboxylase adenovirus or the drug triacsin C on glucose-stimulated insulin secretion argues against the long-chain acyl CoA hypothesis of glucose sensing (12). However, these studies can be justifiably criticized because the parental INS-1 cells used exhibited only 2- to 4-fold responses to glucose, which are well below the 15-fold response of normal islets. Furthermore, based on the work described herein, we now know that parental INS-1 cells have a very limited K_{ATP} channel-independent pathway of glucose sensing, and regulation by long-chain acyl CoAs could occur primarily via this pathway. The robust glucose-stimulated K_{ATP} channel-dependent and -independent responses coupled with the clear potentiating effect of free fatty acids suggest that the new cell lines will be useful for more rigorous and relevant testing of this and other models in the future.

The new 832/13 cell line differs in some fundamental respects from normal islets. First, the cells begin to respond to glucose at a slightly lower threshold concentration (4 mmol/l) than normal rat islets (5.5 mmol/l) and are also maximally responsive at 8 mmol/l glucose, whereas normal rat islets continue to respond to concentrations of ≥15 mmol/l. Second, unlike normal islets, 832/13 cells do not exhibit potentiation of glucose-stimulated insulin secretion by carbachol, a muscarinic receptor agonist. One possible explanation for the shift in glucose responsiveness in 832/13 cells relative to normal islets could be the use of RPMI medium containing 11.1 mmol/l glucose to grow the cell lines used in this study. Thus, culturing parental INS-1 cells (27) or human pancreatic islets (28) at elevated glucose levels

(11–25 mmol/l) causes a modest left shift in glucose dose dependence for insulin secretion. Possible mechanisms by which a left shift may occur include a change in the ratio between glucokinase and low K_m hexokinases (27) or altered expression of more global factors such as HNF-1α. A recent study has shown that mice with heterozygous knockout of HNF-1α have a slightly left-shifted glucose dose-response profile compared with normal islets, although islets from the HNF (+/-) animals did maintain responsiveness at glucose concentrations >8 mmol/l (29). The lack of responsiveness to carbachol is more difficult to explain. We have previously shown that carbachol effectively elevates inositol phosphates in INS-1 cells, which provides evidence that muscarinic receptor signaling is normal in these cells (21). Perhaps INS-1 cells have low or absent levels of expression of the atypical protein kinase C isozyme (Z) that has been implicated in carbachol-stimulated insulin secretion in RIN cells (30).

Further investigation will be necessary to understand the mechanisms underlying the differences in phenotype between the new INS-1 lines and normal rat islets. Nevertheless, the INS-1–derived cell lines described herein appear to be an attractive new tool for investigating metabolic signaling mechanisms in islet β-cells. Future studies will focus on understanding the molecular mechanisms responsible for the subtle differences in glucose responsiveness between the new INS-1 cell lines and normal rat islets and the more pronounced differences between the strongly responsive and poorly responsive INS-1 cell clones.

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Cell Biology

ATCC® Number:	CRL-1469™ Order this Item	Price:	$279.00
Designations:	PANC-1		
Depositors:	M Lieber		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
Source:	**Organ:** pancreas **Tissue:** duct **Disease:** epithelioid carcinoma		
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)		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 11 D16S539: 11 D5S818: 11,13 D7S820: 8,10 THO1: 7,8 TPOX: 8,11 vWA: 15		
Cytogenetic Analysis:	Chromosome studies indicate a modal number of 63 with 3 distinct marker chromosomes and a small ring chromosome. This is a hypertriploid human cell line. The modal chromosome number was 61, occurring in 32% of cells., However, cells with 63 chromosomes also occurred at a high frequency (22%). The rate of cells with higher ploidies was 8.5%.		
Isoenzymes:	G6PD, B		
Age:	56 years		
Gender:	male		
Ethnicity:	Caucasian		

Related Links ▸

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Login Required ▸

- Product Information Sheet

BioProducts

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

BioServices

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

Cell Biology

ATCC® Number:	**CRL-1492™** Order this Item	Price:	**$279.00**

Designations:	AR42J
Depositors:	NW Jessop
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	epithelial PHOTO
Source:	**Organ:** pancreas **Strain:** Wistar **Tissue:** exocrine **Disease:** tumor
Cellular Products:	amylase and other exocrine enzymes [22185]
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:	insulin, expressed glucocorticoid, expressed
Tumorigenic:	Yes
Comments:	Secretory activity is inducible by glucocorticoid stimulation, and is accompanied by extensive re-organization of the endoplasmic reticulum.
Propagation:	**ATCC 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 20% . **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C **Growth Conditions:** The cells cells grow slowly, in clusters. They tend to pile up and appear refractile.

Protocol: Monolayer never becomes confluent. Subculture when

Related Links ▸

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required ▸

Product Information Sheet

BioProducts

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

BioServices

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

invitrogen™

Material Safety Data Sheet

Revision Date: 16-Dec-2005

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

Product code R70507
Product name 293A Cell Line

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

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Dimethyl Sulfoxide	67-68-5	5-10

3. HAZARDS IDENTIFICATION

Emergency Overview
Irritating to eyes. Irritating to skin. Components of the product may be absorbed into the body through the skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes Irritating to eyes.
Skin Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation May cause irritation of respiratory tract.
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 Eyes. Skin.

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	Avoid contact with skin and eyes.
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Dimethyl Sulfoxide	-	-	-	-

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 under normal conditions.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Dimethyl Sulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/
Potential Health effects

Eyes Irritating to eyes.
Skin Irritating to skin. Components of the product may be absorbed into the body through the skin.
Inhalation May cause irritation of respiratory tract.
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 Eyes. Skin.

12. ECOLOGICAL INFORMATION

Ecotoxicity effects No information available.
Mobility No information available.
Biodegradation Inherently biodegradable.
Bioaccumulation Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Dimethyl Sulfoxide	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Dimethyl Sulfoxide	-	-	-	-	-

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Not determined

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.

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

End of Safety Data Sheet

invitrogen™

Material Safety Data Sheet

Revision Date: 10-Jul-2007

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

Product code C0035C
Product name HUVEC, 500,000 cells/vial

Company/Undertaking Identification

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

INVITROGEN CORPORATION
3 FOUNTAIN DRIVE
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
011 44 141 814 6100

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
1-800-263-6236

CASCADE BIOLOGICS
INVITROGEN CORPORATION
1341 S.W. CUSTER DRIVE
PORTLAND, OR 97219
++1 503-292-9521
++1 800-778-4770

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	7-13

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

3. HAZARDS IDENTIFICATION

3. HAZARDS IDENTIFICATION

Emergency Overview
Components of the product may be absorbed into the body through the skin
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Suspension

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	Moderate skin irritation. Components of the product may be absorbed into the body through the skin.
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	1
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Rinse mouth.
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Water spray. Carbon dioxide (CO2). Foam. Dry powder. alcohol-resistant foam. The product is not flammable.
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. Clean contaminated surface thoroughly. Take up mechanically and collect in suitable container for disposal.

7. HANDLING AND STORAGE

Handling Avoid contact with skin and eyes.
Storage Keep in properly labelled containers.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

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 Suspension

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 soluble

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

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	Moderate skin irritation. Components of the product may be absorbed into the body through the skin.
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

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

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 contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

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.

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

End of Safety Data Sheet

LAWSON
HEALTH RESEARCH INSTITUTE

See Toxin Info

375 South Street
London, Ontario N6A
4G5
CANADA

Telephone 519.667.6649
Fascimile 519.432.7367
lawsonresearch.com

FAX TRANSMISSION

TO: Jennifer Stanley
DATE: MARCH 21, 2011
FAX: (519) 661-3420
PAGES: 21 + cover sheet
FROM: GAIL RYDER
SUBJECT: DR. Wang's BARF and MSDS

Hi Jennifer.

Please find BARF + STZ and Tamoxifen MSDS.

Her lab A5-116 will be re-certified on

MARCH 29, 2011. Would have been sooner but I have been waiting for one of the P.I.'s on that floor to be Director for all the TC rooms.

Thx
Gail
x75109

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 4.1
Revision Date 10/20/2010
Print Date 03/14/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Streptozocin
Product Number	:	S0130
Brand	:	Sigma
Product Use	:	For laboratory research purposes.
Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA
Manufacturer	:	Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+19058299500
Fax	:	+19058299292
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Pancreas., Liver, Kidney, Blood, Reproductive system.Pancreas., Liver, Kidney, Blood, Reproductive system.

WHMIS Classification

D2A	Very Toxic Material Causing Other Toxic	Carcinogen
D2B	Effects	Mutagen

GHS Classification

Carcinogenicity (Category 1B)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H350	May cause cancer.

Precautionary statement(s)

P201	Obtain special instructions before use.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

HMIS Classification

Health hazard: 0
Chronic Health Hazard: *
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : *N*-(Methylnitrosocarbamoyl)-α-D-glucosamine
Streptozotocin

Formula : $C_8H_{15}N_3O_7$
Molecular Weight : 265.22 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Streptozocin			
18883-66-4	242-646-8	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

hygroscopic Store under inert gas. Moisture sensitive. Keep in a dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder
Colour	light yellow

Safety data

pH	no data available
Melting/freezing point	Melting point/range: 121 °C (250 °F) - dec.
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available

Vapour pressure	no data available
Density	no data available
Water solubility	soluble
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents, Strong acids, Strong bases

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 5,150 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
Laboratory experiments have shown mutagenic effects.

Genotoxicity in vitro - Human - Kidney
DNA damage

Genotoxicity in vitro - rat - Liver
Unscheduled DNA synthesis

Genotoxicity in vitro - Hamster - Lungs
Sister chromatid exchange

Genotoxicity in vivo - rat - Oral
DNA damage

Genotoxicity in vivo - rat - Intraperitoneal
Unscheduled DNA synthesis

Carcinogenicity

Possible human carcinogen

IARC:	2B - Group 2B: Possibly carcinogenic to humans (Streptozocin)
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
Vomiting

Synergistic effects
no data available

Additional Information
RTECS: LZ5775000

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects
no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3077 Class: 9 Packing group: III
Proper shipping name: Environmentally hazardous substances, solid, n.o.s. (Streptozocin)
Reportable Quantity (RQ): 1 lbs
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

DSL Status
All components of this product are on the Canadian DSL list.

WHMIS Classification

D2A	Very Toxic Material Causing Other Toxic	Carcinogen
D2B	Effects	Mutagen

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH

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Material Safety Data Sheet

Version 4.3
Revision Date 01/06/2011
Print Date 03/14/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Tamoxifen
Product Number	:	T5648
Brand	:	Sigma
Product Use	:	For laboratory research purposes.
Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA
Manufacturer	:	Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+19058299500
Fax	:	+19058299292
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Eyes, Liver, Kidney, Blood

WHMIS Classification

D2A	Very Toxic Material Causing Other Toxic Effects	Carcinogen

GHS Classification

Acute toxicity, Oral (Category 5)
Carcinogenicity (Category 1B)
Reproductive toxicity (Category 1B)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H303	May be harmful if swallowed.
H350	May cause cancer.
H360	May damage fertility or the unborn child.

Precautionary statement(s)

P201	Obtain special instructions before use.
P308 + P313	IF exposed or concerned: Get medical advice/ attention.

HMIS Classification

Health hazard:	1
Chronic Health Hazard:	*
Flammability:	0
Physical hazards:	1

NFPA Rating

Health hazard:	1
Fire:	0
Reactivity Hazard:	1

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : (Z)-1-(p-Dimethylaminoethoxyphenyl)-1,2-diphenyl-1-butene
trans-2-[4-(1,2-Diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine

Formula : $C_{26}H_{29}NO$ $C_{26}H_{29}NO$

Molecular Weight : 371.51 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Tamoxifen			
10540-29-1	234-118-0	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

Light sensitive.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Safety glasses with side-shields conforming to EN166 Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting/freezing	Melting point/range: 97 - 98 °C (207 - 208 °F) - lit.

point	
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
Light.

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx)
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
LD50 Oral - rat - 4,100 mg/kg

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity

no data available

Carcinogenicity

This is or contains a component that has been reported to be carcinogenic based on its IARC, OSHA, ACGIH, NTP, or EPA classification.

Possible human carcinogen

IARC:	1 - Group 1: Carcinogenic to humans (Tamoxifen)
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

May cause reproductive disorders.

Teratogenicity

Presumed human reproductive toxicant

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Synergistic effects
no data available

Additional Information
RTECS: KR5919600

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment

no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

DSL Status
All components of this product are on the Canadian DSL list.

WHMIS Classification

D2A	Very Toxic Material Causing Other Toxic Effects	Carcinogen

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Streptozotocin
Proposed Use Dose:	75000 µg
Proposed Storage Dose:	1000000 µg
LD_{50} (species):	50000 µg

Calculation:

50000 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 2500000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = *250000*

Comments/Recommendations:

Store in two separate locations.

???

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Tamoxifen
Proposed Use Dose:	1000 µg
Proposed Storage Dose:	1000000 µg
LD_{50} (species):	4100000 µg

Calculation:

4100000 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 205000000

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 20500000

Comments/Recommendations:

LD 50 Oral - rat - 4,100mg/kg