THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 25, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: Frank S. Prato
SIGNATURE: [signature]
DEPARTMENT: LHRI
ADDRESS: 268 Grosvenor St., London, ON N6A 4V2
PHONE NUMBER: X 64140
EMAIL: prato@lawsonimaging.ca

Location of experimental work to be carried out: Building(s) SJHC Room(s) F4-127a, F5-119

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: See attached for grants, title and summaries
GRANT TITLE(S):

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Donna Goldhawk
Kim Blackwood
Andrea Mitchell
Wendy Zhu
Andrew McKennan
Salman Ahmed

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

1.0 Microorganisms

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

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

_____

_____

_____

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

_____

_____

_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
E. coli. Strain DH5α	O Yes ⊘No	O Yes ⊘No	O Yes ⊘No	1	Invitrogen	⊘1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*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? ⊘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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ⊘No		Not applicable
Rodent	O Yes ⊘No		
Non-human primate	O Yes ⊘No		
Other (specify) canine	⊘Yes O No	blood, bone marrow	2007-126

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☑ Yes ○ No	MDA-MB-435	I. Karopatnick
Rodent	☑ Yes ○ No	N2A, C2C12, H9C2	S. Dhanvantari; ATCC
Non-human primate	○ Yes ☑ No		
Other (specify)	○ Yes ☑ No		

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

2.4 For above named cell types(s) indicate HC or CFIA containment level required ☑ 1 ☑ 2 ○ 3 Level 1 just

3.0 Use of Human Source Materials

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

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

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

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli	pUC 19 pCR II	Invitrogen	MagA	1.3 kb added

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES ⊘NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used mouse, dog

6.3 AUS protocol # 2008-076-06, 2007-126

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

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7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

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

9.0 Insects Requiring CFIA Permits

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

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ 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

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will the agent be imported? O YES, please give country of origin_____ ⊘ NO
If no, please proceed to Section 10.0

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE _____

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13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
◉ YES, permit # if on-campus Dhanvantari Culture Room - Level 2
○ NO
⊘ NOT REQUIRED For this research

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: Feb 19, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Oct 15/2009

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

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

Special Conditions of Approval:

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

Research Area: Myocardial Therapeutics
Imaging for Cardiovascular Therapeutics

SHSC: Project #9
Investigators: Alexander Dick, Chuck Cunningham
Title: Evaluation of new cell-based therapies with MRI.
Research Area: Myocardial Therapeutics – Regenerative Medicine
Primary Clinical Target Work: Angiogenic therapy in myocardium/heart failure.
Technical Tools: Cell delivery, MR imaging modality.

LHRI: Project #1
Investigators: Donna Goldhawk, Savita Dhanvantari, Terry Thompson, Robert Stodilka, Michael Kovacs, Gerry Wisenberg, Frank Prato
Collaborators: Sandy Dick, Graham Wright, Marc Ruel, Erik Suuoronen
Title: Myocardial Cell Therapy for Heart Failure
Research Area: Myocardial Therapeutics – Regenerative Medicine
Development of a MRI reporter probe to image molecular events in cells transplanted into the myocardium.
Primary Clinical Target Work: Heart failure is predicted by WHO to be the disease with the greatest negative impact on productivity worldwide by 2020. Heart transplantation is the only partially effective therapy, but limited in availability. Regeneration of heart tissue through stem cell transplantation shows promise but methods are needed to image the transplanted cells and determine their viability, molecular activity and engraftment.
Technical Tools: Reporter probes have been developed for optical imaging (e.g. bioluminescence, green fluorescent protein) and nuclear imaging (^{18}F-FHBG for PET and ^{123}I-FIAU for SPECT). However optical has very poor depth penetration and nuclear has poor spatial localization. Here we propose to develop a reporter probe system for MRI, which will have both depth penetration and excellent spatial localization.

LHRI: Project #2
Investigators: Stergios Stergiopoulos, Liang Song, Jianxin Han, Fan Zhang, Frank Prato, James White
Collaborators: Gerry Wisenberg, Ali Islam
Title: Monitoring for Myocardial Therapeutics
Research Area: Myocardial Therapeutics
Development of a complete cardiac ultrasound volumetric imaging system for commercialization.
Primary Clinical Target Work: Vulnerable plaque, valves, occlusive vascular disease, ischemic heart disease
Technical Tools: Cardiac 4D Volumetric Ultrasound Imaging

LHRI: Project #3
Investigators: Ting-Yim Lee, Gerry Wisenberg, Len Luyt, Michael Kovacs
Collaborators: Rob deKemp, Rob Beanlands
Title: Quantitative imaging of sympathetic nervous function of the heart with CT and PET
Research Area: Myocardial Therapeutics
Primary Clinical Target Work: Cardiac sympathetic nervous system contributes to and may be primary responsible for the morbidity and mortality associated with cardiac conditions such as sudden cardiac death and congestive heart failure. A major limitation to understanding the role of sympathetic nervous system in these conditions has been the absence of quantitative methods for non-invasive evaluation of global and regional sympathetic nervous function in the heart. We will develop methods for quantitative imaging of sympathetic nervous function of the heart with a 64-slice CT scanner and PET scanner.
Technical Tools: The intent of the project is to develop software to analyze data from both the CT scanner and PET scanner of a hybrid CT/PET scanner to arrive at measures of sympathetic nerve receptor/transmitter density. We will develop a comprehensive model to describe the delivery of a PET tracer (e.g. Meta-hydroxyephedrine) for the sympathetic nervous systems of the heart by blood flow, transcapillary leakage and uptake at specific receptors. With the data acquired by the CT scanner and PET scanner, the software will produce maps of myocardial blood flow and receptor/transmitter densities.

LHRI: Project #4
Investigators: Robert Stodilka, Frank Prato, Gerald Wisenberg, Michael Kovacs
Collaborators:
Title: Myocardial SPECT/CT
Research Area: Myocardial Therapeutics

We are examining regenerative cell therapy to combat the progressive tissue remodeling that results from cardiac ischemia and recover heart function. In an established large animal model of ischemia/reperfusion, we will monitor the efficacy of progenitor cell transplantation within the injured heart using gene-based cellular contrast and MRI. Our approach is predicated upon strategies used by magnetotactic bacteria, in which we genetically engineer myocyte precursors to form magnetosome-like vesicles. These compartmentalize iron biominerals and function as endogenous contrast agents, conferring magnetic field sensitivity to the cell. This technology will circumvent the shortcomings of superparamagnetic iron oxide (SPIO) nanoparticles, which provide neither functional molecular information nor long-term labelling. In addition, magnetosome gene expression vectors will be developed for reporter gene expression to improve cell tracking with respect to number, location, migration and differentiation. This application is relevant to many fields of regenerative medicine, to stem cell biology, and to commercialization opportunities similar to that of the green fluorescent protein expression vectors.

Grant Applications are Strictly Confidential

CANCER IMAGING NETWORK OF ONTARIO (CINO) RESEARCH GRANT APPLICATION

Application Deadline – January 31, 2007

Title of Research	**Non-invasive Molecular Imaging of Cancer Growth and Metastasis Using MRI and a Novel Reporter Gene**		
Principal Investigator(s), and their locations (List as many investigators/ supervisors as applicable. Separate entries with a hard return)	Frank S. Prato Jim D. Koropatnick Savita Dhanvantari		
Collaborators, and their locations (List as many as required. Separate entries with a hard return)	Donna Goldhawk Robert Z. Stodilka		
Budget Requested	Year 1	Year 2	Budget guidelines – see INFO document for full details
a) Trainee Stipends	48,813	50,453	$22,600 *pa* MSc / $23,600 *pa* PhD / $35,750 *pa* Post-doctoral or clinical fellows
b) Operating Funds	19,000	17,500	Maximum of $15,000 per year per trainee
c) Small Equipment	0	0	For initial purchase of small equipment or software; copies of quotations are required
d) Travel Costs	2,000	2,000	Maximum of $2,000 per year per trainee
Subtotal By Year	**69,813**	**69,953**	Maximum of $70,000 per year per project
TOTAL BUDGET REQUESTED	**$139,766**		

OVERVIEW OF RESEARCH

Explain the objective(s), relevance to CINO objectives, hypothesis, methods, and impact of the research. **Use 300 words or less.**

OBJECTIVES: Magnetic resonance imaging (MRI) is an imaging modality particularly suited for tracking metastatic cells *in vivo*. When labelled with superparamagnetic iron oxide (SPIO) nanoparticles to provide contrast, individual cells can be imaged repeatedly with spatial resolution comparable to tissue. However, there are major limitations to labelling cells with SPIO: the nanoparticles do not report molecular events, MRI is not quantitative, and the SPIO are not retained in rapidly dividing cells. Engineering cells to synthesize endogenous MR contrast would obviate these issues. We propose to develop novel MRI reporter genes that will enable cancer cells to transport and retain iron in a superparamagnetic form. In this way, molecular events in metastasizing cells can be followed *in vivo* over time with MRI.

HYPOTHESES: (1) A novel MRI reporter gene, MagA (in combination with transferrin receptor and ferritin subunits), will enable iron retention in mammalian cancer cells. (2) Metastatic cancer cells expressing MagA can be quantitatively tracked *in vivo* with a combination of MRI and SPECT/CT (single photon emission computed tomography/x-ray computed tomography).

METHODS: We will design reporter constructs for MRI and cancer therapy, using both constitutive and inducible promoters. MagA-expressing cells will be imaged *in vitro* by MRI and SPECT/CT, and analyzed for magnetosome-like structures by microscopy and energy dispersive x-ray. MagA-expressing cells will be labelled with ^{111}Indium-tropolone, injected into mice and quantitatively tracked using both MRI and SPECT/CT. Both CT and MRI images will be co-registered to provide the anatomical link between SPECT radiotracer and signal voids generated by MRI. Images will be correlated to histological analysis of tissue.

IMPACT: This project will demonstrate the use of MagA as a novel reporter gene-based contrast agent for molecular imaging with MRI. Its application in a cancer cell model will provide information about cell localization, migration, proliferation and differentiation.

MRI - Magnetic Viability — the benefit of these imaging techniques on patient mortality,

myocardial infarction, heart failure

Summary of Proposal

Moderate to severe left ventricular dysfunction secondary to coronary artery disease is associated with a very high mortality and morbidity. The annual risk of death, myocardial infarction, and hospitalization for heart failure approaches 50%. These high rates of adverse events are seen in patients with both chronic coronary disease, i.e. the condition known as ischemic cardiomyopathy, and following acute myocardial infarction, when the left ventricular ejection fraction is below 35%. The impairment of left ventricular function may be irreversible, secondary to scar formation. However, in many patients, the abnormalities of regional systolic contraction are reversible, when the ischemic tissue is revascularized, and the myocardium is considered to have been "hibernating". Many studies, using a variety of non-invasive imaging modalities, have demonstrated the ability to predict the myocardium that will benefit most by revascularization. Positron emission tomography using fluorinated de-oxyglucose has emerged as the best tool to use to recognize myocardial hibernation and predict functional improvement. There has been the assumption that in those patients where significant improvement in systolic function occurred, a corresponding improvement in survival and other adverse events would ensue. A recent meta-analysis suggested a near 80% reduction in mortality in those patients where hibernation was identified and who received revascularization with either coronary artery bypass grafting or percutaneous angioplasty in comparison to those treated medically with residual viable, but ischemic myocardium. In those patients where dysfunction was secondary to scar tissue, there was no significant difference between modes of therapy. However, none of the trials making up this meta-analysis were prospectively randomized, with management guided by the outcome of viability imaging.

Recently, contrast enhanced Magnetic Resonance Imaging has become a powerful tool to evaluate the extent of scar formation. It has been used primarily in the setting of acute or recent myocardial infarction, to evaluate the extent of scar formation, and predict functional recovery following myocardial stunning. There have been no studies reported in the literature to date regarding the association of the finding on post-infarction MR scans, and the subsequent incidence of death, myocardial infarction and heart failure, with or without revascularization in these patients. MRI has also been applied only to a limited extent in patients with ischemic cardiomyopathy.

Within Ontario, a consortium of cardiac imaging has been formed with established clinical and investigative expertise in both positron emission tomography and magnetic resonance imaging. This consortium will facilitate a definitive prospective trial to be undertaken to evaluate the role of these sophisticated imaging modalities in potentially helping to reduce the extremely high burden of hard clinical endpoints in patients with coronary artery disease and moderate to severe left ventricular dysfunction.

The trial, with participation from Ottawa, London, and Toronto will randomize a total of 1914 patients with ejection fractions of 35% or less, either following a myocardial infarction, or with a chronic ischemic cardiomyopathy to one of three arms: 1) standard viability assessment with thallium SPECT perfusion imaging or in addition 2) MRI scar imaging or 3) FDG PET viability imaging Subsequent management will be guided by the results of the imaging modalities. Patients will be followed for a two-year period to determinate death, infarction and heart failure rates, with serial evaluation of left ventricular function. This trial will be a milestone in assessing the role of these imaging modalities for patients at high risk for adverse events.

Towards Image Guided Myocardial Stem Cell Therapy: Molecular Imaging, Nanotechnology and Stem Cell Biology

Summary of Research Proposal

Background And Overall Goal: Coronary artery disease, resulting in heart failure, is the leading cause or a leading cause of death worldwide. Functioning heart tissue is replaced by non-function scar, as the permanently damaged myocardial tissue does not regenerate. Present methods of therapy are inadequate with the most effective form in many cases being heart transplantation. Limited supply and immune rejection made this therapy of "last resort" wholly inadequate. In 2003 in the US alone more than 550,000 new cases of heart failure were diagnosed, but only some 2,000 transplantations performed. (In Canada there are 70,000 new cases diagnosed each year with 4500 deaths and only 150 to 180 heart transplants performed.) One potential therapy, although presently controversial, is the transplantation of stem cells; preferably autologous. Almost all of the research investigating myocardial stem cell therapy has been carried out in small animal, rodent, models. Even though the result of this research has been controversial, the clinical need is so great that human clinical testing has begun. The "Holy Grail" of "Image Guided Myocardial Stem Cell Therapy" has the following preferred embodiment: a) harvesting autologous adult stem cells i.e. donor and recipient are identical, b) selecting and programming the adult stem cells, c) transplanting the cells, d) Tracking the cells from a few hours to 10 or more weeks after transplantation to determine cell viability, differentiation and engraftment, e) determining the extent of metabolic and functional recovery

However, a great deal remains to be discovered before stem cell therapy can be optimally applied to humans. Much of this is best done in small animals but work in large animals is also needed. Desperately needed are *in vivo* imaging methods to follow in these large animals, the fate of the transplanted cells. To achieve this goal imaging methods will have to exploit molecular biology technology and nanotechnology. Our well-characterized canine models of coronary artery disease will be used to develop some of the needed technology.

Research Objectives: Two fundamental unanswered questions include a) what cells to transplant and how should they be pre-treated and b) where, when and how should they be transplanted. The answers to these two questions could be more quickly resolved if a) there was a relevant large animal model and b) transplanted cells in a large animal model and in humans could be non-invasively followed (imaged) and characterized with respect to viability, differentiation and engraftment. The large animal model is needed to address issues of a) autologous transplantation in which donor and recipient are the same individual, b) delivery methods in which the absolute infarct volume is more representative of the human patient, and c) defining the therapeutic window between the initial inflammation following the ischemic event and the formation of permanent scar. Further, large animal non-invasive cell tracking methods can be more easily adapted for use in human studies than small animal methods.

Our team have two interdependent objectives that will evolve through an iterative research plan made possible by the team formation. First, we will develop *in vivo* non-invasive molecular and cellular imaging methods to track transplanted cells. Then, with some of this technology in hand, we will begin the investigation of stem cell transplantation methodologies including: a) different cell fractions, b) delivery methods and c) timing in relation to the evolution of heart failure. The image tracking methods will need to adapt as stem cell therapy discoveries will, in many cases, require altered cell labelling methodologies.

Interactions and Collaborations of Team Members: We propose herein to network six independently funded laboratories, three in London Ontario and three in Toronto Ontario, to develop the needed large animal model (canine) and the needed large animal/human non-invasive imaging methods. Dr. M.

Hough's canine stem cell biology laboratory (Toronto) will collaborate with stem cell molecular biologists (working with Dr. F. Prato in London) to select the cells and "program" them. Dr. G. Wisenberg's laboratory (London) and Dr. A. Dick's (Toronto), will develop in the canine model cell delivery methods and Drs. G. Wright (Toronto) and F. Prato will develop (along with a new investigator) the MRI cell tracking nanotechnology and Drs. G. Wells and a second new investigator will develop the SPECT and PET cell tracking technology. Progression in the two overall objectives (development of the canine myocardial stem cell model and the cell tracking imaging methods) is interdependent. For example, a reporter gene/reporter probe PET imaging method may work for one cell population but not for another. Hence, what needs to be developed are core technologies and competencies which, once developed, can be used repeatedly and quickly to advance the overall goal of myocardial stem cell therapy. The following disciplines will be incorporated into the core competencies: canine stem cell biology, radiation dosimetry, cell physiology (cell viability, proliferation and differentiation assays and functional integration of donor cells in recipient cardiac tissue), molecular imaging (nuclear medicine reporter gene/reporter probe), nanotechnology (MRI iron-contrast agents), radiochemistry (PET & SPECT reporter probes), veterinarian cardiology and cardiac surgery, MRI imaging, SPECT & PET imaging, and optical imaging (cell culture and excised tissue analysis).

Research Plan: Team members will select the somatic and embryonic stem cells that have demonstrated *in vitro* and via organogenesis the greatest potential to survive and differentiate into myocardial tissue cell types. These will be transplanted into canine models of infarction and heart failure. (The canine model is selected over the swine model as the pre-existence of collaterals provide a more representative model of the development of acute myocardial infarction in patients with chronic, slowly progressing coronary disease.) The cell tracking imaging methods already developed will be used to evaluate transplanted cell survival and location over the first two weeks for both somatic and embryonic stem cells. Investigators will focus on route of delivery (peripheral vein, coronary artery and myocardial injections), time between infarction and transplantation, number of cells to be transplanted, and pharmacology treatment (e.g. immune suppression for ES cells, soluble factors that stimulate stem cell differentiation including possible stimulation of endogenous cardiac stem cells). The imaging technologies will be further refined to distinguish differentiation and engraftment of transplanted stem cells from differentiation of endogenous stem cells. This will be done in Toronto first by applying nanotechnology to MRI and molecular stem cell biology to SPECT and PET. MR technology will be developed to allow cell number quantification as well as localization. The SPECT and PET molecular imaging technology will be developed to allow cell number quantification, detection of differentiation of stem cells to cardiac cells and successful engraftment of the regenerated tissue. We will address: a) question of radiotoxicity (deterministic and stochastic risks) and iron oxide toxicity, b) development of co-labelling strategies, c) development of tracking methods in canine organogenesis and d) translation of tracking methods to human patients.

Value Added: The core competencies in molecular and cellular imaging and in canine myocardial stem cell therapy can only be achieved by effective interaction and collaboration of all six team members. Dr. M. Hough provides the knowledge of which stem cell to transplant; Dr. A. Dick provides the methodology of MR image guided stem cell transplantation; Dr. G.W. Wright provides the methodology of iron oxide nanotechnology; Dr. G. Wells provides the SPECT and PET technology; Dr. G. Wisenberg the canine model of coronary artery disease; Dr. F. Prato the development of reporter probes for SPECT, PET and possibly MRI. Each laboratory has independent funding and a strong track record in training and innovation of imaging and stem cell biology. Hence, there is significant added value in multi-disciplinary training of new investigators, postdoctoral fellows and graduate students. Once reality, these core competencies will attract new team members (see collaboration of support letters) including small animal researchers wishing to test results in large animal and clinical trial researchers with desire to test procedures to be used on humans.

Imaging in Ischemic Heart Disease: Making Diagnosis, Following Therapy

Coronary artery disease (CAD) is the single largest cause of death in the world. Since the late 1980's, we have been developing MRI methods to improve the detection of permanently damaged heart tissue, i.e. infarcted myocardium as separate from normal and reversibly damaged tissue. To validate and understand the physiological basis of these imaging methods, we developed and characterized canine models of CAD. These models have proved so effective that they have also been used to investigate, as funded by other sources, therapies designed to limit tissue damage following myocardial ischemia. It has recently been hypothesized by us that these large animal models are ideal to bridge the gaps between research in rodents and clinical application in the new emerging areas of myocardial stem cell therapy. Over the next 5 years we propose to 1) continue to improve on the MRI methods to quantitate the extent of ischemic heart disease in both dogs and humans, and 2) contribute to the emerging area of image guided myocardial stem cell therapy by developing non-invasive cell tracking technologies in our large animal model using SPECT, PET as well as MRI.

Our short-term goal is to 1) further improve the sensitivity of myocardial viability MR imaging and, 2) further develop quantitative myocardial blood flow imaging. This work is well underway in our canine models of coronary artery disease and now it is important that some of these results be validated in patients post myocardial infarction. Hence, we have started a study in patients where MR imaging is performed in the acute period after a heart attack (within 3 weeks) and then 6 months later. This is allowing us to understand the uptake and washout kinetics of Gd-DTPA, a MR contrast agent, and how these vary as the infarct ages in both reperfused infarcted myocardium and non-reperfused infarcted tissue.

Our longer-term goal is to develop, in our large animal model, new non-invasive imaging methods (using MRI, SPECT, and PET) to follow the fate of transplanted stem cells. Our preliminary data in dogs and work done in rodents by a collaborator suggests that the following are realistic objectives over the next 5 years. 1) Track (using SPECT) for up to two weeks the location and number of transplanted autologous stem cells, 2) Track over 10 weeks or more the viability, engraftment and differentiation of the transplanted stem cells (using SPECT and gene probe/reporter probe molecular imaging technology adapted from small animal work) and, 3) Transfer the technology from a SPECT platform to a PET one to reduce radiation dose to the transplanted cells and their progeny in preparation for human trials.

Success in achieving these goals will contribute to the development of human image guided myocardial stem cell therapy.

SUMMARY OF RESEARCH PROPOSAL

TITLE: MECHANISMS BY WHICH LOW FREQUENCY MAGNETIC FIELDS INTERACT WITH BIOLOGICAL SYSTEMS

We propose to continue our work elucidating the mechanisms by which relatively weak (<1 mT), low frequency (<3000Hz) magnetic fields produce biological effects. We have contributed substantially to scientific knowledge in two important areas: a) we have established reproducible biological effects in humans and b) we have contributed to knowledge regarding the initial biophysical transduction event and the cascade of events that precede physiological outcomes as studied in humans, animals and cells. In addition, we have performed work in humans, rodents, human cells and biochemical reactions toward a third goal: the observation of biological effects *during* magnetic field exposure.

In humans, we will investigate the importance of exposure parameters of the pulsed magnetic field. It now seems clear that the initial detection/transduction step is a necessary but not sufficient condition to elicit a physiological/behavioural response. This work will be primarily performed using EEG brain mapping and fMRI. We will test our working pulsed magnetic field design assumptions that have already allowed us to (a) alter standing balance (in patients and in normals), and (b) report pain levels (in patients and normals as well as in animals), and anxiety (in patients as well as in animals). Initial data indicates that both EEG and fMRI can monitor brain activation during magnetic field exposure. Also, we will determine if the initial biophysical detection event in humans is related to the induced current and/or the scalar and/or the direction of the applied field.

In rodents, we will investigate: a) the minimum magnetic field parameters that can cause a behavioural/physiological response, b) the role of visible light in magnetic field detection and relation to final behavioural/physiological event, and c) the dose relationship between pulsed magnetic field parameters, behavioural/physiological response and biochemical markers of neuronal activity.

In human cells, we will measure the effects of magnetic field exposure on cytosolic calcium concentrations, during the exposure itself, in order to determine the influence of cell state and media conditions, as well as the relationship between dose and magnetic field parameters. In a cell free peroxidase/oxidase oscillating biochemical reaction, we will evaluate the importance of magnetic and visible light parameters on shifting the oscillating phase and frequency.

We intend to apply knowledge regarding mechanisms (from detection to behavioural/physiological response) to: a) minimizing unwanted side effects particularly from patient MRI exposures and b) maximizing therapeutic applications.

invitrogen™

Material Safety Data Sheet

Revision Date: 09-Nov-2006

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

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

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 %
Glycerol	56-81-5	5-10

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

3. HAZARDS IDENTIFICATION

Emergency Overview

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects No information available

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
Glycerol	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
Glycerol	Listed	-	Listed	-	Listed

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

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

Western

Jan. 1, 2009
***This is the 1st Renewal of this protocol**
*A Full Protocol submission will be required in **2011**

Dear Dr. **Prato:**

Your Animal Use Protocol form entitled:

Evaluation Using SPEC/CT of Free 111-In Injected into Normal Myocardium - Pilot

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **Jan. 1, 2009 to Dec. 31, 2009**

The protocol number for this project remains as **2007-126**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - F. Prato, J. Sykes, D. Forder
Approval Letter - F. Prato, J. Sykes, D. Forder

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
PH: 519-661-2111 ext. 86770 • FL 519-661-2028 • www.uwo.ca / animal

Western

June 25, 2008

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2008

Dear Dr. Koropatnick:

Your Animal Use Protocol form entitled:
Non-invasive Molecular Imaging of Cancer Growth and Metastasis Using MRI and a Novel Reporter Gene
Funding Agency CANCER IMAGING NETWORK OF ONTARIO - Grant #LRI7767116-4951000

has been approved by the University Council on Animal Care. This approval is valid from **June 24, 2008 to June 30, 2009.** The protocol number for this project is **#2008-076-06**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 1 Year

Species	Strain	Other Detail	Pain Level	Animal # Total for 1 Year
Mouse	CD-1 nu/nu	6 tp 8 weeks Female	C	100

STANDARD OPERATING PROCEDURES
Procedures in this protocol should be carried out according to the following SOPs. Please contact the Animal Use Subcommittee office (661-2111 ext. 86770) in case of difficulties or if you require copies.
SOP's are also available at http://www.uwo.ca/animal/acvs
100 Monitoring/Tumour Growth/Rodents
310 Holding Period Post-Admission
320 Euthanasia
321 Criteria for Early Euthanasia/Rodents
330 Post-Operative Care/Rodent
343 Surgical Prep/Rodent/Recovery Surgery

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - J. Koropatnick, D. Goldhawk, T. Kirkpatrick
Approval Letter - D. Goldhawk, T. Kirkpatrick

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
PH: 519-661-2111 ext. 86770 • FL 519-661-2028 • www.uwo.ca / animal

From: "Kathy Floyd" <kfloyd@uwo.ca>
Subject: 2008-076-06 Koropatnick new animal protocol
Date: Tue, July 15, 2008 9:48 am
To: "Patt Brocksom" <brocksom@uwo.ca>,"Sherry Paiva [Sherry.Paiva@LHSC.ON.CA]" <Sherry.Paiva@LHSC.ON.CA>
Cc: "James Koropatnick" <jkoropat@uwo.ca>,"Donna Goldhawk" <dgoldhawk@lawsonimaging.ca>

Hi :

Dr. J. Koropatnick, Oncology, UWO/LRCP has a new animal protocol entitled "Non-invasive Molecular Imaging of Cancer Growth and Metastasis Using MRI and a Novel Reporter Gene"
has been approved as of June 25th, 2008.

The Funding for this project is from:

CANCER IMAGING NETWORK OF ONTARIO
Non-invasive Molecular Imaging of Cancer Growth and Metastasis Using MRI and a Novel Reporter Gene - LRI7767116-4951000

Thanks
Kath

Attachments:

untitled-1	
Size:	0.4 k
Type:	text/plain

Cell Biology

ATCC® Number:	**CRL-1446™** Order this Item	Price:	**$256.00**
Designations:	H9c2(2-1)	Depositors:	W Carlisle
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)	Morphology:	myoblast

Source:
Strain: BD1X
Organ: heart
Tissue: myocardium

Cellular Products: myokinase; creatine phosphokinase; myosin

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.

Related Cell Culture Products

Applications: transfection host(Roche FuGENE® Transfection Reagents)

Receptors: acetylcholine, expressed

Age: embryo

Comments: H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue by B. Kimes and B. Brandt and exhibits many of the properties of skeletal muscle.
Myoblastic cells in this line will fuse to form multinucleated myotubes and respond to acetylcholine stimulation.
Fusion occurs faster if the serum concentration in the medium is reduced to one percent.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Protocol: The myoblastic population will become depleted rapidly if the cultures are allowed to become confluent.
To prevent loss of myoblastic cells, cultures should be subcultured before they become confluent, and the line should be recloned periodically with selection for myoblastic cells.

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the

flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

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

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:
1062: Kimes BW, Brandt BL. Properties of a clonal muscle cell line from rat heart. Exp. Cell Res. 98: 367-381, 1976. PubMed: 943302
32970: Levy AP, et al. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. J. Biol. Chem. 271: 2746-2753, 1996. PubMed: 8576250

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Cell Biology

ATCC® Number:	**HTB-129™**	Order this Item	Price:	**$264.00**

Designations:	MDA-MB-435S
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	spindle shaped 
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion
Cellular Products:	tubulin; actin
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.
Isolation:	**Isolation date:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2

Related Links

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

	PGM1, 2 PGM3, 1
Age:	31 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	This cell line was originally described as a spindle shaped variant of the parental MDA-MB-435 strain isolated in 1976 by R. Cailleau, et al. from the pleural effusion of a 31 year old female with metastatic, ductal adenocarcinoma of the breast. However, recent studies have generated questions about the origin of the parent cell line, MDA-MB-435, and by extension HTB-129. Gene expression analysis of the cells produced microarrays in which MDA-MB-435 clustered with cell lines of melanoma origin instead of breast [PubMed ID: 10700174, PubMed ID: 15150101, PubMed ID: 15679052]. Additional studies have since corroborated a melanocyte origin of MDA-MB-435, to which ATCC has responded by pursuing its own investigation into the identity of this cell line. The cell line to which MDA-MB-435 is reported to have been cross-contaminated with is the M14 melanoma line [PubMed ID: 12354931 and PubMed ID: 17004106]. **Derivatives of HTB-129 with identities in question:** M4A4, ATCC ® CRL-2914 M4A4 GFP, ATCC ® CRL-2915 M4A4 LM3-2 GFP, ATCC ® CRL-2916 M4A4 LM3-4 CL 16 GFP, ATCC ® CRL-2917 NM2C5, ATCC ® CRL-2918 NM2C5 GFP, ATCC ® CRL-2919
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml insulin; fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 100% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, add fresh 0.25%trypsin - 0.53 mM EDTA, rinse and remove. Place flask at room temperature (or incubated at 37C) for approximately 10 minutes or until the cells detach. Add fresh medium, aspirate and dispense into new flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Culture medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	purified RNA:ATCC HTB-129R purified DNA:ATCC HTB-129D Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008 recommended serum:ATCC 30-2020
	1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 40: 3118-3129, 1980. PubMed: 7000337 22429: Siciliano MJ, et al. Mutually exclusive genetic

References:

signatures of human breast tumor cell lines with a common chromosomal marker. Cancer Res. 39: 919-922, 1979. PubMed: 427779
22656: Cailleau R, et al. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro 14: 911-915, 1978. PubMed: 730202
32341: Sheng S, et al. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996. PubMed: 8876194
32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: 8650224
49803: Ross DT, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nature Genetics 24: 227-235, 2000. PubMed: 10700174
89918: Ellison G, et al. Further evidence to support the melanocytic origin of MDA-MB-435. Mol. Pathol. 55: 294-299, 2002. PubMed: 12354931
90826: Sellappan s, et al. Lineage infidelity of MDA-MB-435 cells: expression of melanocyte proteins in a breast cancer cell line. Cancer Res. 64: 3479-3485, 2004. PubMed: 15150101
90828: Rae JM, et al. Common origins of MDA-MB-435 cells from various sources with those shown to have melanoma properties. Clin. Exp. Metastasis 21: 543-552, 2004. PubMed: 15679052

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Cell Biology

ATCC® Number:	**CCL-131™**	Order this Item	Price:	**$256.00**

Designations:	Neuro-2a
Depositors:	RJ Klebe
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	neuronal and amoeboid stem cells 
Source:	**Strain:** A **Organ:** brain **Disease:** neuroblastoma **Cell Type:** neuroblast;
Cellular Products:	acetylcholinesterase tubulin
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)
Virus Susceptibility:	Herpes simplex virus Vesicular stomatitis virus Human poliovirus 1
Reverse Transcript:	negative
Antigen Expression:	H-2, a haplotype; Mus musculus, expressed
Cytogenetic Analysis:	modal number = 95; range = 59 to 193. Karyotype unstable within a stemline range of 94 to 98 chromosomes. All the cells contain 6 to 10 large chromosomes with median or submedian centromeres and 2 to 4 minute chromosomes.
GenoType:	albino
Comments:	Clone Neuro-2a was established by R.J. Klebe and F.H. Ruddle from a spontaneous tumor of a strain A albino mouse. This tumor line, designated C1300, was obtained from the Jackson Laboratory, Bar Harbor, Maine [22161].Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells.The cell line has been used for studies on the mechanism of vinblastine precipitation of microtubular protein, the kinetics of GTP binding to isolated protein, the turnover of microtubules in vivo, and the synthesis and assembly of microtubular protein [PubMed: 5263744]. The World Organization for Animal Health (OIE) uses the cells for

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

routine diagnosis of rabies. (see: http://www.oie.int/Eng/Normes/Mmanual/A_00044.htm)Tested and found negative for ectromelia virus (mousepox).

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Protocol:

Subculturing:

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

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 1 to 2 times per week

Preservation:

Freeze medium: Complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

1023: Olmsted JB, et al. Isolation of microtubule protein from cultured mouse neuroblastoma cells. Proc. Natl. Acad. Sci. USA 65: 129-136, 1970. PubMed: 5263744
22161: Klebe RJ, Ruddle FH. Neuroblastoma: Cell culture analysis of a differentiating stem cell system. J. Cell Biol. 43: 69A, 1969.
29352: Naslavsky N, et al. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J. Biol. Chem. 272: 6324-6331, 1997. PubMed: 9045652
29861: Kaneko K, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94:

10069-10074, 1997. PubMed: 9294164
32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

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Cell Biology

ATCC® Number:	**CRL-1772™**	Order this Item	Price:	**$256.00**

Designations:	C2C12
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	myoblast 
Source:	**Strain:** C3H **Tissue:** muscle **Cell Type:** myoblast;
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)
Comments:	This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903] The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953] Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427] Tested and found negative for ectromelia virus (mousepox).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Protocol:** IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT. Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture. Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

Related Links

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

Subculturing:

3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2.
6. Incubate cultures at 37°C.

Preservation:

Medium Renewal: Every two to three days

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22903: Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524

22953: Blau HM, et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846

23427: Katagiri T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [published erratum appears in J Cell Biol 1995 Feb;128(4):following 713]. J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324

28236: Chow YH, et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336

32828: Kessler PD, et al. Gene delivery to skeletal muscle results in sustanined expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064

33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

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Product Description

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Cell Biology

ATCC® Number: CRL-1446™ Order this Item
Price: $256.00

Designations: H9c2(2-1)
Depositors: W Carlisle
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: Rattus norvegicus (rat)
Morphology: myoblast
Source: **Strain:** BD1X
Organ: heart
Tissue: myocardium
Cellular Products: myokinase; creatine phosphokinase; myosin
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: acetylcholine, expressed
Age: embryo
Comments: H9c2(2-1) is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue by B. Kimes and B. Brandt and exhibits many of the properties of skeletal muscle.
Myoblastic cells in this line will fuse to form multinucleated myotubes and respond to acetylcholine stimulation.
Fusion occurs faster if the serum concentration in the medium is reduced to one percent.
Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

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

Subculturing:

Protocol: The myoblastic population will become depleted rapidly if the cultures are allowed to become confluent.
To prevent loss of myoblastic cells, cultures should be subcultured before they become confluent, and the line should be recloned periodically with selection for myoblastic cells.

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

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

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

Related Products:

recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

1062: Kimes BW, Brandt BL. Properties of a clonal muscle cell line from rat heart. Exp. Cell Res. 98: 367-381, 1976. PubMed: 943302
32970: Levy AP, et al. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. J. Biol. Chem. 271: 2746-2753, 1996. PubMed: 8576250

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Material Safety Data Sheet

Revision Date: 13-Feb-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARAT ION AND THE COMPANY/UNDERTAK ING

Product code 54357
Product name pUC 19 Control DNA

Company/Undertak ing Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
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/I NFORMATION ON INGREDIENTS

Hazardous/Non-ha zardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

3. HAZARDS IDENTIFICATION

Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures — Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice

Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

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

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

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

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Material Safety Data Sheet

Revision Date: 17-Apr-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARAT ION AND THE COMPANY/UNDERTAK ING

Product code 460801
Product name pCR2.1-TOPO

Company/Undertak ing Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
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/I NFORMATION ON INGREDIENTS

Hazardous/Non-ha zardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	50
Triton X-100	9002-93-1	0.1

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

3. HAZARDS IDENTIFICATION

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

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

HMIS

Health	No Information Available
Flammability	No Information Available
Reactivity	No Information Available

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-
Triton X-100	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)
Triton X-100	1800 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects No information available

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

Target Organ Effects — No information available

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
Glycerol	Listed	Listed	Listed	Listed	-	Listed
Triton X-100	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
Glycerol	Listed	-	Listed	-	Listed
Triton X-100	-	-	-	-	-

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

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Material Safety Data Sheet

Revision Date: 09-Apr-2009

1. IDENTIFICATION OF THE SUBSTANCE/PREPARAT ION AND THE COMPANY/UNDERTAK ING

Product code 500313
Product name EC TOP10, ONE SHOT

Company/Undertak ing Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

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

2. COMPOSITION/I NFORMATION ON INGREDIENTS

Hazardous/Non-ha zardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	7-13

3. HAZARDS IDENTIFICATION

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

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/
Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

7. HANDLING AND STORAGE

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

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

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)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	No information available.
Bioaccumulation	No information available

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
Glycerol	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
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

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

16. OTHER INFORMATION

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

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End of Safety Data Sheet