

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	<u>Thomas A. Drysdale</u>
DEPARTMENT	<u>Paediatrics</u>
ADDRESS	<u>A5-138, 5th Floor VRL</u>
PHONE NUMBER	<u>519-685-8500 ext. 55072</u>
EMERGENCY PHONE NUMBER(S)	<u>519 473-1458</u>
EMAIL	<u>tadrysda@uwo.ca</u>

Location of experimental work to be carried out: Building(s) 5th Floor VRL Room(s) A5-118, A5-129

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, NSERC
GRANT TITLE(S): CIHR - Molecular Control of Cardiac Morphogenesis and Differentiation
NSERC - Molecular Basis of Thyroid and Lung Development

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Thomas A. Drysdale</u>	<u>tadrysda@uwo.ca</u>	<u>June 27 2006</u>
<u>Jean Wang</u>	<u>jwang423@uwo.ca</u>	<u>June 12, 2010</u>
<u>Meaghan Melling</u>	<u>mmellin@uwo.ca</u>	<u>Dec. 8 2009</u>

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

We use two strains of *Escherichia coli* for the growth and manipulation of plasmid DNA: E. coli. XL1 Blue and DH5 α . We maintain stocks of competent cells of these two strains and they are grown on agar plates or liquid cultures when generating plasmid. Agar plates are disposed of by autoclaving and liquid cultures are killed with bleach.

We use three mammalian cell lines to test the function of specific proteins or signaling pathways on the expression of specific genes. At the present time we use HaCaT cells as these are responsive to both the bone morphogenetic protein and retinoic acid signaling pathways. These cultures are stored as stocks in a -180°C freezer. We then grow them in room A5-118 using standard cell culture techniques. They are transfected with specific plasmids using lipid-based transfection reagents. The cells are then harvested for either protein or RNA. All culture dishes and pipettes used to handle the cells are autoclaved after use. We are not currently using the NIH/3T3 or HEK-293 cell lines but we do have stocks stored at -180°C.

Please include a one page research summary or teaching protocol.

CIHR

Objective: The long-term objective of this proposal is to understand how specific signaling pathways control the differentiation and morphogenesis of the cardiovascular system.

Governing Hypothesis: *Establishment of the various cell lineages within the heart is accomplished by a series of patterning events where external cues specify or maintain subdomains within a single, multipotential heart field.* This patterning gives rise to the differences in morphogenetic properties within the heart field with the anterior domain remain multipotential and having migratory properties and the posterior domain differentiating earlier and being able to form a tube.

Approach: We will use both pharmacological and molecular approaches to alter either specific signaling pathways or specific genes in whole *Xenopus* embryos. Changes in cardiovascular development, will be analyzed by comparing morphology and the expression of genes that mark specific regions of the heart after these treatments. Other biochemical approaches will be used to test specific interactions between signaling systems.

Specific Objective 1: Determine the role of opposing FGF and RA signaling gradients in the patterning and morphogenesis of the early heart field. Our lab has demonstrated that there is significant early patterning of the lateral plate mesoderm (LPM) and that opposing gradients of retinoic acid (RA) and Fibroblast growth factor (Fgf) signaling are critical in that patterning event. A similar opposing gradient covers the developing heart field and we propose to determine how those gradients are responsible for the gene expression patterns, differentiation properties, and the morphogenesis properties that distinguish the anterior and posterior ends of that heart field. Discovery of novel cardiac markers that are regulated by RA or Fgf will be done using a microarray-based approach.

Specific Objective 2. Determine the how the larger LPM region is patterned and the role of early transcription factors in that patterning. We propose that understanding patterning of the LPM into myocardial, endothelial and smooth muscle lineages will give insight into how putative cardiogenic progenitor differentiation is controlled. We will test this in whole embryos by looking at the differentiation of the three lineages and also test the functional role of the early transcription factors that we have described as regional markers of the LPM.

Specific Objective 3. Determine the interactions with other key signaling systems in cardiac development. We propose that Fgf and RA signaling directly interact with the Bone morphogenetic pathway and Notch signaling in defining the early heart field and have identified potential mechanisms for those interactions. We will test those mechanistic models using whole embryo and cell-based biochemical approaches.

Specific Objective 4. To better elucidate the role of Shroom3 in cardiac development. One of the key differences between the anterior and posterior heart field is that only the posterior heart field forms a tube. To understand this, we have investigated how a tube is formed in the early heart and found that Shroom3 is necessary for tube formation. Investigating what controls Shroom3 is investigated in Objective 1 and in this objective, we will determine how Shroom3 regulates tube formation in order to gain a wider knowledge base for understanding cardiac morphogenesis.

Summary: This proposal will begin to understand how the heart field is patterned and how that pattern is important in establishing patterns of gene expression and the morphogenesis of the heart.

NSERC

Endoderm is a term that describes the interior layer of an early embryo. This layer will generate a simple tube that will give rise to many important organs including the thyroid, lung, pancreas, liver, and stomach. These organs arise from specific points along the tube in a precise spatial order. The thyroid comes from the very front of the tube and the lung comes from endoderm located further back. At the earliest stages of development, these two regions share many genes that are essential for their eventual development and so we wish to discover what makes the two organs different. We have found that addition of retinoic acid, the active form of vitamin A, causes the thyroid to turn off several important thyroid genes and turn on genes that are normally only expressed in lung. Our goal is to understand this switch in developmental fate. We will also exploit this switch to search for new genes that important for making a thyroid or a lung. Once we have obtained genes that are expressed in one organ or the other, we will manipulate those genes to examine their effect on thyroid and lung differentiation. By doing so we hope to gain a better understanding of the molecular network needed for the normal development of these endodermal organs.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? YES NO

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

What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli – DH5 α	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	100 ml. liquid		X 1 2 2+ 3
E. coli – XL1-Blue	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	100 ml. liquid		X1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO

If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="checkbox"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	X Yes No	HEK-293, HaCaT	2	Local colleagues
Rodent	X Yes No	NIH/3T3	2	Local colleagues
Non-human primate	O Yes X No			
Other (specify)	O Yes X No			

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Blood (fraction) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Organs or Tissues (unpreserved)		Yes Unknown		O 1 O 2 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? X 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 from transformation or tranfection
<i>E. coli</i> DH5α <i>E. coli</i> XL1 Blue	XPax-2a XNkx2.1 XEtsrp cGFP-rGBD Xhoxc10 Xror2 Islet1 XPox2	A. Brandli P. Krieg P. Krieg W.M. Bement C. Niehrs M. Taira D. Melton T. Mohun	<i>Xenopus Pax-2</i> <i>Xenopus Nkx2.1</i> <i>Xenopus Etsrp</i> eGFP fused to RhoA binding domain of rhotekin <i>Xenopus Hoxc10</i> <i>Xenopus Ror2</i> <i>Xenopus Islet1</i> <i>Xenopus Pox2</i>	For all of these plasmids there are no visible changes to the bacteria except that it provides antibiotic resistance due to the normal plasmid backbone.

	<i>XNkx2.5</i> <i>XNkx2.3</i> <i>XSM1</i>	<i>P. Krieg</i> <i>P. Krieg</i> <i>P. Thiebaud</i>	<i>Xenopus Nkx2.5</i> <i>Xenopus Nkx2.3</i> <i>Xenopus MHC</i> <i>SM1 isoform</i>	
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* Please attach a Material Data Sheet or equivalent if available.

** Please attach a plasmid map.

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Will live animals be used? YES No

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

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

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES X 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 LD₅₀ (specify species) of the toxin _____

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

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES X NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
X YES, date of most recent biosafety inspection: April 5, 2011
NO, please certify
NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-LHRI-0070

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
There are no risks that are unique to the agents listed. These are very standard laboratory reagents and the use of level 2 guidelines is more than sufficient to essentially eliminate any minor risk associated with their use.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
If exposed to the E. coli cultures, washing of the affected area with soap and water will be done. Spilling of the cell cultures would result in a similar response of thorough cleaning of the affected area. There are no circumstances where work with these reagents would involve use of a syringe.

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

SIGNATURE  Date: July 6 2011

15.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: 
Date: July 7, 2011

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

Special Conditions of Approval:

Appendix 1.

MSDS Sheets for agents listed.

Note, we have not been able to find an MSDS sheet for the HaCaT cells. We are assuming that because they are both immortal human keratinocyte lines that the MSDS sheet for HEK-293 cells will be applicable.



Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biohazards et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2



By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are considered to be level 1 animal pathogens:

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

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

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

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
 1600 FARADAY AVE.
 CARLSBAD, CA 92008
 760/603-7200

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

INVITROGEN CORPORATION
 3 FOUNTAIN DR.
 INCHINNAN BUSINESS PARK
 FAISLEY, PA4 9RF
 SCOTLAND
 44-141 814-6100

INVITROGEN CORPORATION
 P.O. BOX 12-502
 PENROSE
 AUCKLAND 1135
 NEW ZEALAND
 64-9-579-3024

INVITROGEN CORPORATION
 2270 INDUSTRIAL ST.
 BURLINGTON, ONT
 CANADA L7P 1A1
 905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
 800/451-8346 (24 HOUR)
 800/955-6288

NON-EMERGENCY INFORMATION:

Product Name: LIBRARY EFFICIENCY DH5ALPHA COMPETENT CELLS
 Stock Number: 18263012

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
 These products are for laboratory research use only and are not intended for human or animal diagnostics, therapeutic, or other clinical uses.

Synonyms:
 Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
DIMETHYL SULFOXIDE	67-68-5	3 - 7

MATERIAL SAFETY DATA SHEET

LIBRARY EFFICIENCY DH5ALPHA COMPETENT CELLS	Page 2 of 8
INVITROGEN CORPORATION	Revised 9/30/03
MSDS ID: 18263	Replaces 9/05/03
	Printed 9/30/03

3. HAZARDS IDENTIFICATION

***** EMERGENCY OVERVIEW *****
 Warning!
 Irritant.
 Harmful if absorbed.

Potential Health Effects:

Eye: Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin: Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage. Upon prolonged or repeated exposure, harmful if absorbed through the skin. May cause minor systemic damage.

Inhalation: Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache. No toxicity expected from inhalation.

Ingestion: Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

Chronic:
 No data on cancer.

4. FIRST AID MEASURES

Eye: Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin: Wash with soap and water. Get medical attention if irritation develops or persists.

Inhalation: Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion: Do not induce vomiting and seek medical attention immediately. Drink two

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LIBRARY EFFICIENCY DHSALPHA COMPETENT CELLS	Revised 9/30/03
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4. FIRST AID MEASURES (CONT.)

glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

- Flashpoint Deg C: Not available.
- Upper Flammable Limit %: Not available.
- Lower Flammable Limit %: Not available.
- Autoignition Temperature Deg C: Not available.

Extinguishing Media:
Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Carbon dioxide Carbon monoxide Sulfur containing gases

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

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6. ACCIDENTAL RELEASE MEASURES (CONT.)

Spill Cleanup:
 Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill. Ventilate the contaminated area. Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
 Ambient

Handling Procedures:

Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area. Keep closed or covered when not in use.

Storage Procedures:

Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed. Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
DIMETHYL SULFOXIDE	Not established.	Not established.

Engineering Controls:

Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
 Safety glasses should be the minimum eye protection. Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash

MATERIAL SAFETY DATA SHEET

LIBRARY EFFICIENCY DH5ALPHA COMPETENT CELLS INVITROGEN CORPORATION MSDS ID: 18263	Page 5 of 8 Revised 9/30/03 Replaces 9/05/03 Printed 9/30/03
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8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Gloves should be used as minimum hand protection.

Respiratory:
Use supplied-air respiratory equipment as required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state: Liquid solution / suspension
Odor: No odor.

Not established.
Not established.

Specific Gravity/Density: Not established.
Octanol/water Partition Coeff: Not established.
Volatiles: Not established.
Evaporation Rate: Not established.
Viscosity: Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalis. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO

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10. STABILITY AND REACTIVITY (CONT.)

degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Sulfur containing gases.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG

Target Organs: Blood. Eyes. Skin.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades slowly.

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13. DISPOSAL CONSIDERATIONS

Regulatory Information:
 Not applicable.

Disposal Method:
 Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
 Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
 Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
 This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
 No Prop 65 Chemicals.
 No 313 Chemicals

CANADA:

DSL/NDSL:
 Not determined.

COMPONENT DIMETHYL SULFOXIDE WHMIS Classification D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES: None assigned.
 PRODUCT SAFETY PHRASES: Not applicable.
 PRODUCT CLASSIFICATION:

MATERIAL SAFETY DATA SHEET

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15. REGULATORY INFORMATION (CONT.)

Not classified

Component
DIMETHYL SULFOXIDE

EINECS
Number
200-664-3

16. OTHER INFORMATION

HMS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

Abbreviations

N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMSIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and
Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial
Chemical Substances

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 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 PARTICULAR PURPOSE.

Material Safety Data Sheet



XL1-Blue Electroporation-Competent Cells, Catalog #200228

1. Product and company identification

Product name : XL1-Blue Electroporation-Competent Cells, Catalog #200228

Material uses : Analytical reagent.

pUC18 Control Plasmid DNA 0.01 ml
 XL1-Blue electroporation competent cells 0.5 ml (0.1 ml / Tube)

Supplier/Manufacturer : Agilent Technologies, Inc.
 1834 State Highway 71 West
 Cedar Creek, TX 78612

Part No. (Chemical Kit) : 200228

Part No. : pUC18 Control Plasmid DNA 200231-42
 XL1-Blue electroporation competent cells 200228-41

Validation date : 04/04/2011

In case of emergency : 1-800-894-1304

2. Hazards identification

Physical state : pUC18 Control Plasmid DNA Liquid.
 XL1-Blue electroporation competent cells Liquid.

Odor : pUC18 Control Plasmid DNA Not available.
 XL1-Blue electroporation competent cells Not available.

OSHA/HCS status : pUC18 Control Plasmid DNA While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.

XL1-Blue electroporation competent cells This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Emergency overview

Signal word : pUC18 Control Plasmid DNA No signal word.
 XL1-Blue electroporation competent cells No signal word.

Hazard statements : pUC18 Control Plasmid DNA NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

XL1-Blue electroporation competent cells MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

Precautions :

2. Hazards identification

	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	XL1-Blue electroporation competent cells	Avoid breathing vapor or mist. Avoid contact with eyes. Avoid prolonged or repeated contact with skin. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.
Routes of entry	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
<u>Potential acute health effects</u>		
Inhalation	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. Slightly irritating to the respiratory system.
Ingestion	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Skin	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. Slightly irritating to the skin.
Eyes	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. Slightly irritating to the eyes.
<u>Potential chronic health effects</u>		
Chronic effects	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. Contains material that may cause target organ damage, based on animal data.
Carcinogenicity	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Mutagenicity	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Teratogenicity	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Developmental effects	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Fertility effects	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No known significant effects or critical hazards. No known significant effects or critical hazards.
Target organs	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
<u>Over-exposure signs/symptoms</u>		
Inhalation	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. Adverse symptoms may include the following: respiratory tract irritation coughing
Ingestion	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. No specific data.

2. Hazards identification

Skin	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. Adverse symptoms may include the following: irritation redness
Eyes	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. Adverse symptoms may include the following: irritation watering redness
Medical conditions aggravated by over-exposure	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	None known. Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (Section 11)

3. Composition/information on ingredients

Name	CAS number	%
XL1-Blue electroporation competent cells		
Glycerol	56-81-5	5 - 10

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

4. First aid measures

Eye contact	: pUC18 Control Plasmid DNA	Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.
	XL1-Blue electroporation competent cells	Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.
Skin contact	: pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if symptoms occur.
	XL1-Blue electroporation competent cells	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention immediately.
Inhalation	: pUC18 Control Plasmid DNA	Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms occur.
	XL1-Blue electroporation competent cells	Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention

4. First aid measures

		immediately.
Ingestion	: pUC18 Control Plasmid DNA	Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms occur.
	XL1-Blue electroporation competent cells	Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention immediately.
Protection of first-aiders	: pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training.
	XL1-Blue electroporation competent cells	No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.
Notes to physician	: pUC18 Control Plasmid DNA	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.
	XL1-Blue electroporation competent cells	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

5. Fire-fighting measures

Flammability of the product	: pUC18 Control Plasmid DNA	In a fire or if heated, a pressure increase will occur and the container may burst.
	XL1-Blue electroporation competent cells	In a fire or if heated, a pressure increase will occur and the container may burst.
Extinguishing media		
Suitable	: pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
	XL1-Blue electroporation competent cells	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	: pUC18 Control Plasmid DNA	None known.
	XL1-Blue electroporation competent cells	None known.
Special exposure hazards	: pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training.
	XL1-Blue electroporation competent cells	No action shall be taken involving any personal risk or without suitable training.
Hazardous thermal decomposition products	: pUC18 Control Plasmid DNA	No specific data.
	XL1-Blue electroporation competent cells	Decomposition products may include the following materials: carbon dioxide carbon monoxide
Special protective equipment for fire-fighters	: Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	

6. Accidental release measures

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

7. Handling and storage

Handling	: pUC18 Control Plasmid DNA	Put on appropriate personal protective equipment (see Section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas.
	XL1-Blue electroporation competent cells	Potentially biohazardous material. Put on appropriate personal protective equipment (see Section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. Do not ingest. Avoid contact with eyes, skin and clothing. Avoid breathing vapor or mist. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Keep in the original container or an approved alternative

7. Handling and storage

made from a compatible material, kept tightly closed when not in use. Empty containers retain product residue and can be hazardous. Do not reuse container.

Storage : pUC18 Control Plasmid DNA

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

XL1-Blue electroporation competent cells

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

8. Exposure controls/personal protection

Ingredient	Exposure limits
XL1-Blue electroporation competent cells Glycerol	<p>ACGIH TLV (United States, 2/2010). TWA: 10 mg/m³ 8 hour(s). Form: Inhalable fraction. See Appendix C, paragraph A. Inhalable Particulate Mass TLVs (IPM-TLVs) for those materials that are hazardous when deposited anywhere in the respiratory tract.</p> <p>OSHA PEL (United States, 6/2010). TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust</p> <p>OSHA PEL 1989 (United States, 3/1989). TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust</p>

Recommended monitoring procedures : If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.

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

Hygiene measures : Handle as biohazard material (Biosafety level 1). Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal protection

8. Exposure controls/personal protection

Respiratory	: Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Hands	: Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Eyes	: Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.
Skin	: Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
Environmental exposure controls	: Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.
Other protection	: Not available.

9. Physical and chemical properties

Physical state	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Liquid. Liquid.
Flash point	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Auto-ignition temperature	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Flammable limits	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Color	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Odor	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
pH	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	7.5 Not available.
Boiling/condensation point	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	100°C (212°F) Not available.
Melting/freezing point	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	0°C (32°F) Not available.
Specific gravity	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Vapor pressure	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.

9. Physical and chemical properties

Vapor density	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Volatility	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Odor threshold	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Evaporation rate	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Viscosity	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Not available. Not available.
Solubility	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Easily soluble in the following materials: cold water and hot water. Easily soluble in the following materials: cold water and hot water.

10. Stability and reactivity

Chemical stability	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	The product is stable. The product is stable.
Conditions to avoid	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. No specific data.
Materials to avoid	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	No specific data. No specific data.
Hazardous decomposition products	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Under normal conditions of storage and use, hazardous decomposition products should not be produced. Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Possibility of hazardous reactions	: pUC18 Control Plasmid DNA XL1-Blue electroporation competent cells	Under normal conditions of storage and use, hazardous reactions will not occur. Under normal conditions of storage and use, hazardous reactions will not occur.

11. Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
XL1-Blue electroporation competent cells Glycerol	LD50 Oral	Rat	12600 mg/kg	-

Irritation/Corrosion

Product/ingredient name	Result	Species	Score	Exposure	Observation
XL1-Blue electroporation competent cells Glycerol	Eyes - Mild irritant Skin - Mild irritant	Rabbit Rabbit	- -	- -	- -

11. Toxicological information

Sensitizer

Conclusion/Summary : Not available.

Chronic toxicity / Carcinogenicity / Mutagenicity / Teratogenicity / Reproductive toxicity

Not available.

Other adverse symptoms : pUC18 Control Plasmid DNA Not available.
 XL1-Blue electroporation Not available.
 competent cells

12. Ecological information

Ecotoxicity : No known significant effects or critical hazards.

Aquatic ecotoxicity

Product/ingredient name	Result	Species	Exposure
XL1-Blue electroporation competent cells Glycerol	Acute LC50 54 to 57 ml/L Fresh water	Fish - Oncorhynchus mykiss - 0.9 g	96 hours

Conclusion/Summary : Not available.

Partition coefficient: n-octanol/water : pUC18 Control Plasmid DNA Not available.
 XL1-Blue electroporation competent cells Not available.

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

13. Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Significant quantities of waste product residues should not be disposed of via the foul sewer but processed in a suitable effluent treatment plant. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Care should be taken when handling emptied containers that have not been cleaned or rinsed out. Empty containers or liners may retain some product residues. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

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

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

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

14. Transport information

Regulatory information

DOT / IMDG / IATA / : Not regulated.

15. Regulatory information

HCS Classification : pUC18 Control Plasmid DNA Not regulated.
 XL1-Blue electroporation competent cells Target organ effects

U.S. Federal regulations : TSCA 8(a) IUR: Partial exemption
 United States inventory (TSCA 8b): All components are listed or exempted.
 SARA 302/304/311/312 extremely hazardous substances: No products were found.
 SARA 302/304 emergency planning and notification: No products were found.
 SARA 302/304/311/312 hazardous chemicals: Glycerol; D-Glucitol
 SARA 311/312 MSDS distribution - chemical inventory - hazard identification:
 Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; D-Glucitol:
 Delayed (chronic) health hazard
 Clean Water Act (CWA) 311: Edetic acid

Clean Air Act Section 112(b) Hazardous Air Pollutants (HAPs) : Not listed

Clean Air Act Section 602 Class I Substances : Not listed

Clean Air Act Section 602 Class II Substances : Not listed

DEA List I Chemicals (Precursor Chemicals) : Not listed

DEA List II Chemicals (Essential Chemicals) : Not listed

State regulations

Massachusetts : The following components are listed: GLYCERINE MIST
 New York : None of the components are listed.
 New Jersey : The following components are listed: GLYCERIN; 1,2,3-PROPANETRIOL
 Pennsylvania : The following components are listed: 1,2,3-PROPANETRIOL
 California Prop. 65

No products were found.

16. Other information

Label requirements : pUC18 Control Plasmid DNA NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
 XL1-Blue electroporation competent cells MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

Date of issue : 04/04/2011
 Date of previous issue : No previous validation.
 Version : 2

Indicates information that has changed from previously issued version.

Notice to reader

Disclaimer: The information contained in this document is based on Agilent's state of knowledge at the time of preparation. No warranty as to its accurateness, completeness or suitability for a particular purpose is expressed or implied.

 Section 1 - Product and Company Information

Product Name 293 CELLS, HUMAN EMBRYO KIDNEY
 Product Number 85120602
 Brand SIGMA

 Company Sigma-Aldrich Canada, Ltd
 Address 2149 Winston Park Drive
 Oakville ON L6H 6J8 CA
 Technical Phone: 9058299500
 Fax: 9058299292
 Emergency Phone: 800-424-9300

 Section 2 - Composition/Information on Ingredient

Substance Name	CAS #		SARA 313
EUROPEAN COLLECTION OF CELL CULTURES, HUMAN SOURCE	None		No
Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
DIMETHYL SULFOXIDE	67-68-5	10	No
CELLS, HUMAN ORIGIN	None	<= 1	No

Chemical Family Human source material.

 Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Biohazard.

Handle as if capable of transmitting infectious agents. Readily absorbed through skin. Target organ(s): Eyes. Skin.

HMIS RATING

HEALTH: 0*

FLAMMABILITY: 0

REACTIVITY: 0

SPECIAL HAZARD(S): Human Source

NFPA RATING

HEALTH: 0

FLAMMABILITY: 0

REACTIVITY: 0

SPECIAL HAZARD(S): Human Source

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

 Section 4 - First Aid Measures

ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE

In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

EYE EXPOSURE

In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT

N/A

AUTOIGNITION TEMP

N/A

FLAMMABILITY

N/A

EXTINGUISHING MEDIA

Suitable: Use extinguishing media appropriate to surrounding fire conditions.

FIREFIGHTING

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Wash spill site with 10% bleach and ventilate area after material pickup is complete.

ENVIRONMENTAL PRECAUTION(S)

Avoid contaminating sewers and waterways with this material.

Section 7 - Handling and Storage

HANDLING

User Exposure: Do not breathe vapor. Avoid prolonged or repeated exposure. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body.

STORAGE

Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator.

Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES

Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance	Physical State: Liquid	
Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.

Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products

not known.

HAZARDOUS EXOTHERMIC REACTIONS

Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.
Skin Absorption: Readily absorbed through skin. May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Eyes. Skin.

SIGNS AND SYMPTOMS OF EXPOSURE

Potentially biohazardous material.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. Disposal should be made in accordance with existing disposal practices employed for infectious waste at your institution. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT

Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

EU ADDITIONAL CLASSIFICATION

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 07/04/2011
Date Updated: 02/04/2006
Version 1.2

Section 1 - Product and Company Information

Product Name NIH 3T3 CELLS, MOUSE SWISS NIH EMBRYO
FIBROBLAST
Product Number 93061524
Brand SIGMA
Company Sigma-Aldrich Canada, Ltd
Address 2149 Winston Park Drive
Oakville ON L6H 6J8 CA
Technical Phone: 9058299500
Fax: 9058299292
Emergency Phone: 800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #		SARA 313
EUROPEAN COLLECTION OF CELL CULTURES, NON-HUMAN / NON-PRIMATE	None		No
Ingredient Name	CAS #	Percent	SARA 313
The hazards identified with this product are those associated with the following component(s):	None		
DIMETHYL SULFOXIDE	67-68-5	10	No

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Readily absorbed through skin. Target organ(s): Eyes. Skin.

HMIS RATING

HEALTH: 0*
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING

HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0

*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is
conscious. Call a physician.

INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give

artificial respiration. If breathing is difficult, give oxygen.

DERMAL EXPOSURE

In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE

In case of contact, immediately flush eyes with copious amounts of water for at least 15 minutes.

Section 5 - Fire Fighting Measures

FLASH POINT

N/A

AUTOIGNITION TEMP

N/A

FLAMMABILITY

N/A

EXTINGUISHING MEDIA

Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Combustible liquid. Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL

Evacuate area.

PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear respirator, chemical safety goggles, rubber boots, and heavy rubber gloves.

METHODS FOR CLEANING UP

Cover with dry-lime, sand, or soda ash. Place in covered containers using non-sparking tools and transport outdoors. Ventilate area and wash spill site after material pickup is complete.

ENVIRONMENTAL PRECAUTION(S)

Avoid contaminating sewers and waterways with this material.

Section 7 - Handling and Storage

HANDLING

User Exposure: Do not breathe vapor. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

STORAGE

Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS

Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator.

Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

Skin-Specific: Chemical resistant apron.

GENERAL HYGIENE MEASURES

Wash contaminated clothing before reuse. Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance	Physical State: Liquid	
Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY

Stable: Stable.

Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Nature of decomposition products not known.

HAZARDOUS EXOTHERMIC REACTIONS

Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Readily absorbed through skin.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Eyes. Skin.

Section 12 - Ecological Information

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT

Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

US CLASSIFICATION AND LABEL TEXT

US Statements: Readily absorbed through skin. Target organ(s): Eyes. Skin.

UNITED STATES REGULATORY INFORMATION

SARA LISTED: No

CANADA REGULATORY INFORMATION

WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.

DSL: No

NDSL: No

Section 16 - Other Information

DISCLAIMER

For R&D use only. Not for drug, household or other uses.

WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale. Copyright 2010 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.

Appendix 2.

Plasmid Maps.

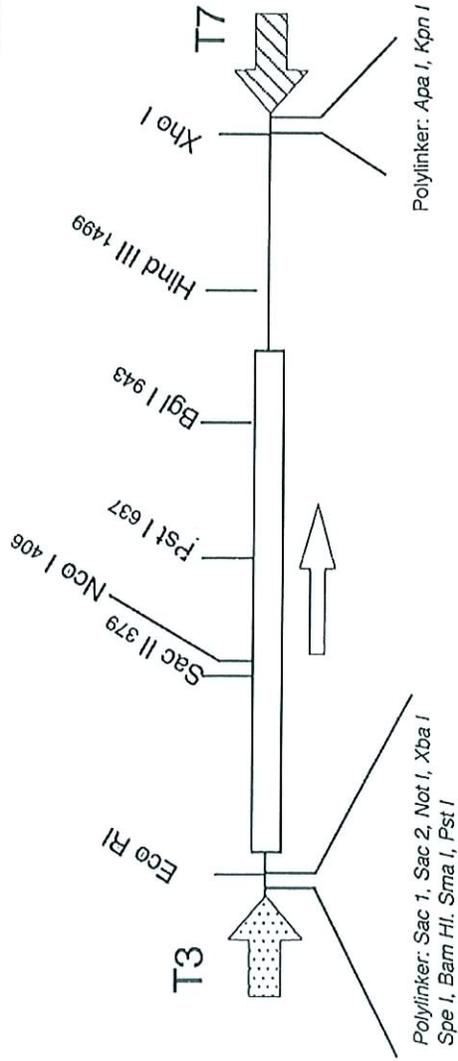
Xenopus Nkx2-1

Eric Small, March 1999.

1614 bp insert in Bluescript SK+.

Paul A. Krieg
Department of Cell Biology and Anatomy
University of Arizona College of Medicine
PO Box 245044
1501 N. Campbell Ave
Tucson AZ 85724

Ph: 520-626-9370
FAX: 520-626-2097
e-mail: pkrieg@email.arizona.edu



Full length cDNA containing the Xenopus Nkx2-1 sequence. Total length of insert is 1614 bp. Restriction site locations are relative to the 5' Eco RI site at +1. The coding region extends from 162 to 1202bp of the clone. Contains a short poly-A tail. For preparation of antisense probe, linearize with Not I or Bam HI and transcribe with T7 RNA polymerase. For expression details, see Small, E.M., Vokes, S.A., Garriock, R.J., Li, D. and Krieg, P.A. (2000) Developmental Expression of the Xenopus Nkx2-1 and Nkx2-4 genes. Mech. Dev. 96, 259-262. Accession number for the sequence is AF281080.

271 XNKX2-1

THE UNIVERSITY OF
ARIZONA[®]
HEALTH SCIENCES CENTER

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College of Medicine
Department of Cell Biology & Anatomy
1501 N Campbell Avenue
PO Box 245044
Tucson AZ 85724-5044
0710140 ROADM

#335

Plasmid name: eGFP-rGBD

Sent from: Bement lab (Department of Zoology - University of Wisconsin, Madison)

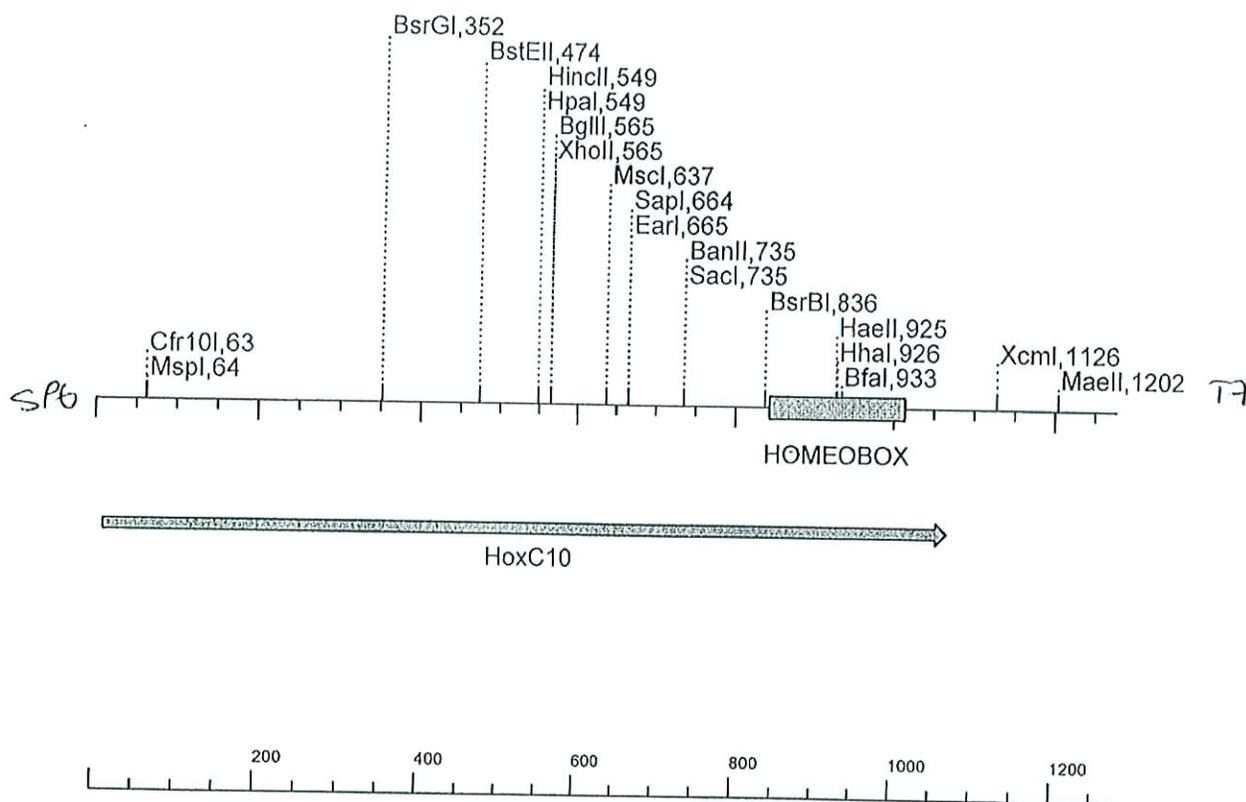
Vector: pCS2

To visualize active RhoA, the RhoA-binding domain of rhothekin (rGBD) fused to eGFP.

rGBD binds specifically to active (GTP bound) RhoA in biochemical assays using cultured mammalian cells (Ren et al., 1999) or *Xenopus* brain extracts (Li et al., 2002).

Cloned using Xho1 and Xba1

Reference: Benink, H.A., and Bement, W.M. (2005). Concentric zones of active RhoA and Cdc42 around single cell wounds. *J Cell Biol* 168, 429-439.



Xhox c10 (1276 bps)

Molecule: Xhox c10, 1276 bps DNA
 File Name: xhoxc10cds.cm5, dated 02 Aug 2002
 Description: CDS in PGMET
 Notes:

347

»|

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User Name Plasmid No **899**

Date 01/10/08 Plasmid Name **XPOX2**

of entry on database
01/10/2008 Gene Names *XPOX2, mpo*

of last modification Species *laevis*

Reference / Gift Smith S., MoD 2002. Tim Mohun Lab,

Sense NotI, Sp6

Antisense SalI, T7

Vector pSport1

Cloning Method and Relevant Information

Describe cloning strategy and purpose of construct

Original cloning by Stuart Smith.2002 paper

Has it been sequenced or verified?

yes no

Sequence

[Link](#)

[Open](#)

Save sequence in AmayaFileStore under databases. Linking not available via web

Map

[Link](#)

[Open](#)

Nkx2-5 and Nkx2-3 in T7TS.

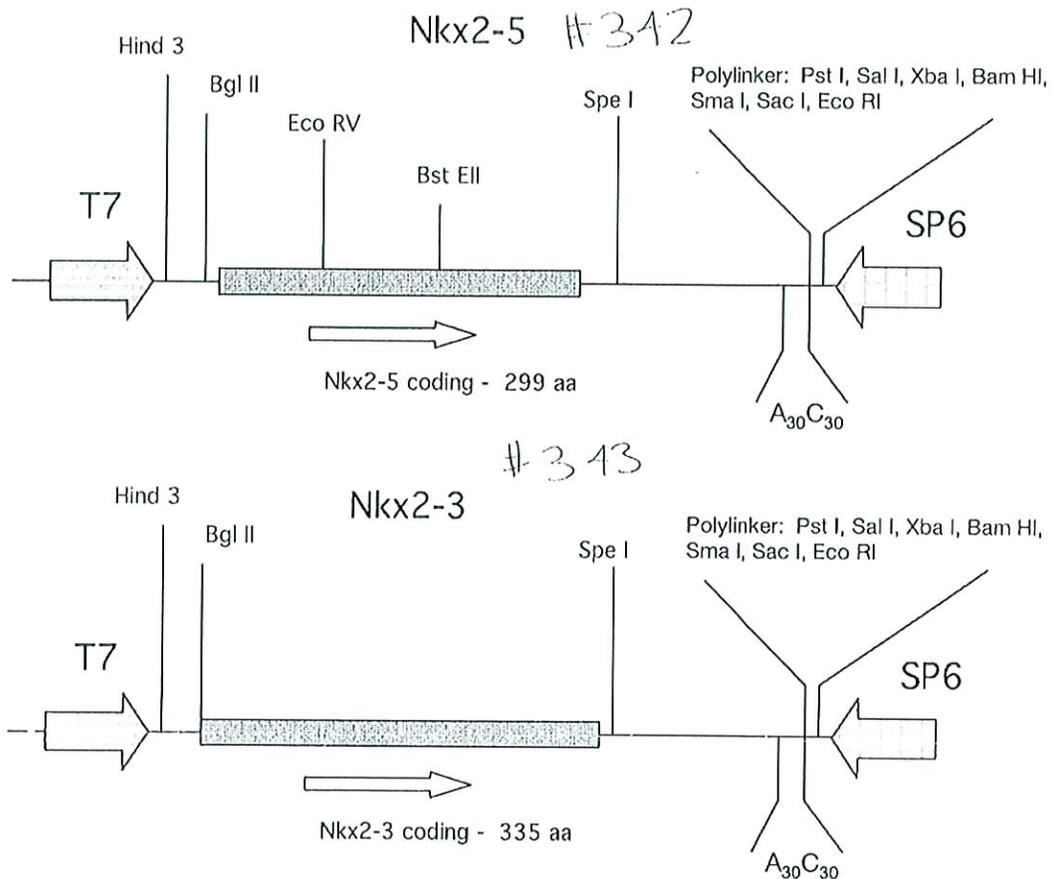
Paul A. Krieg

Department of Cell Biology and Anatomy
 University of Arizona College of Medicine
 1656 E. Mabel Street, MRB 311
 Tucson, Arizona, 85724

Phone: (520) 626-9370

Fax: (520) 626-7600

e-mail: pkrieg@email.arizona.edu



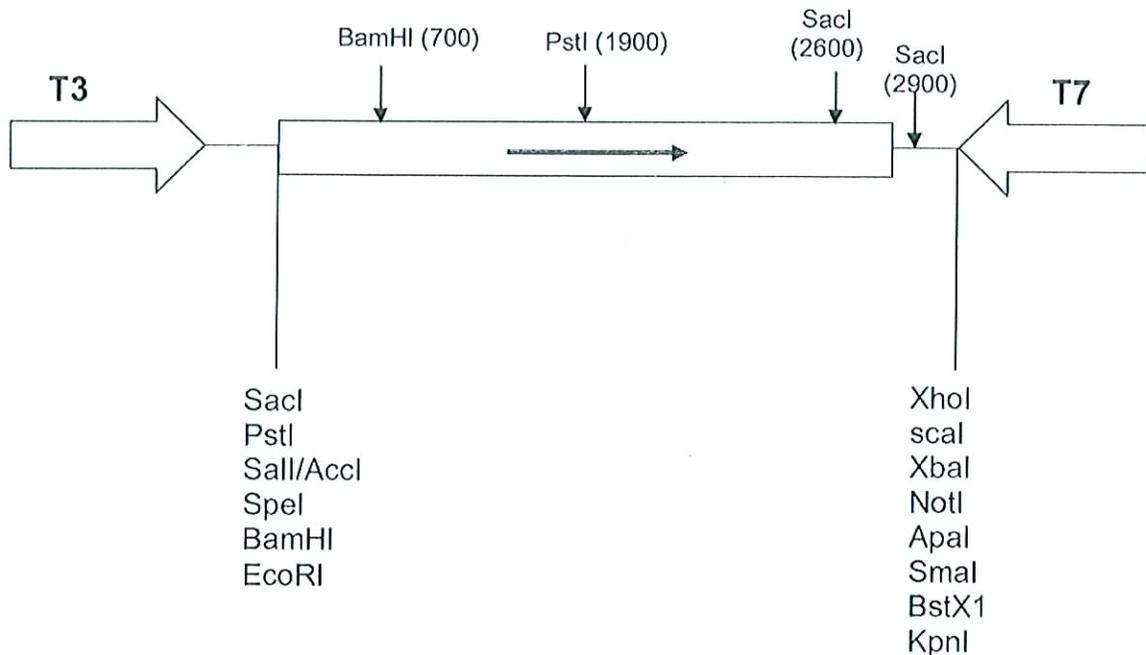
A 1230 bp Kpn1 to Bam HI fragment of the *Xenopus* Nkx2-5 cDNA clone was inserted into the EcoRV site of pT7TS. A 1031 bp PCR fragment containing the coding region of Nkx2-3 was inserted into the EcoRV site of T7TS. In both cases, synthetic mRNA is generated by linearizing with Eco RI and transcribing with T7 RNA polymerase. Referenced in Cleaver et al., (1996). Overexpression of tinman related genes Nkx2-5 and Nkx2-3 in *Xenopus* embryos results in myocardial hyperplasia. *Development* 122, 3549-3556.

#337

.....
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<http://www.umr5164.u-bordeaux2.fr>

XSM1

! Please consider the environment - do you really need to print this email?
.....



XSM1 is a 3176 bp cDNA clone encoding the C-ter region of the *Xenopus laevis* SM-MHC SM1 isoform (aa 1045 to 1973). No sites for EcoRI, XhoI or NotI. The vector is pBK-CMV.

For antisense probe digest with EcoRI and use T7 RNA polymerase.
For sense probe digest with XhoI and use T3 RNA polymerase.

↓
kanamycin (re)

All SM clones from
Pierre Thiébaud.

#355

Date: 23/10/02

Number: 1-34

Made by:

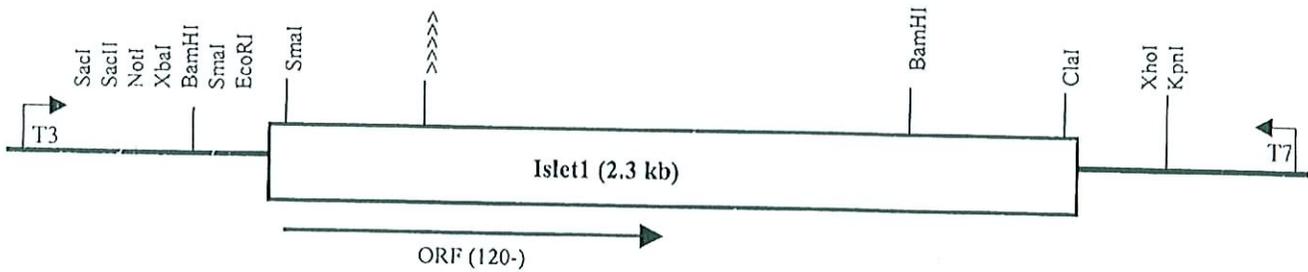
Plasmid Name: Islet1 (5.3 kb)

Vector: pBS SK II (3 kb)

Insert: Xenopus Islet1 (2.3 kb)

Comments: Obtained the cDNA from Melton lab (Kelly). Contains the whole ORF.

Antisense: EcoRI linearize (T7)



Xenopus Laevis Etsrp? pGEM-T Easy (*in situ*)

Cloned by Stryder Meadows

Paul A. Krieg

Department of Cell Biology and Anatomy
University of Arizona College of Medicine
1501 N. Campbell Ave., P.O. Box 245044
Tucson, Arizona, 85724

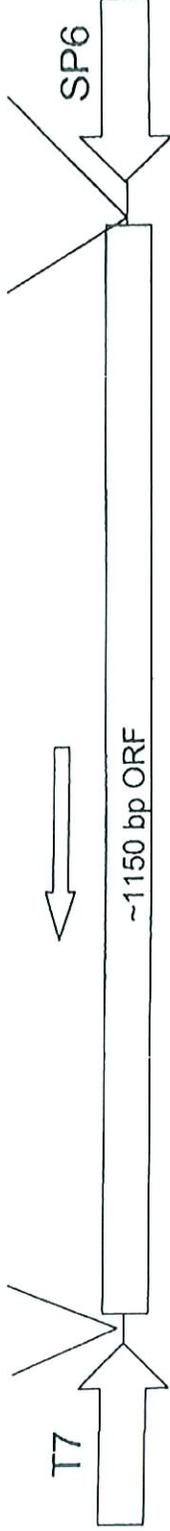
Phone: (520) 626-9370

Fax: (520) 626-2097

e-mail: pkrieg@u.arizona.edu

Apal, Aat II, Sph I, BstZ I, NcoI, BstZ I, Not I, Sac II, EcoRI

Spe I, Eco RI, Not I, BstZ I, Pst I, Sal I, Nde I, Sac I, BstX I, Nsi I



The entire ORF (~1100 bp) of *Xenopus laevis* Etsrp? was PCR amplified with Taq DNA polymerase from a full length EST(BC099054) and TA cloned into pGEM-T Easy.

For *in situ* probe, linearize with SpeI and transcribe with T7.

275 X Pax - 2a

Plasmid name: pBS4 A.3

Plasmid library #: 272

Constructed by: Nicole Heller

Date:

Reference: Heller, N. and Brändli, A.W. (1997) *Mech. Dev.* 69, 83-104.

Insert name: XPax - 2a (1)
 size: 1184 bp
 restriction sites: 5': EcoRI 3': XbaI

Vector name: p Bluescript II SK+
 size: 2958 bp

Host strain: DH5α Selection: Amp

RNA probe synthesis

- antisense

restrict. enzyme: EcoRI
 promotor: T3

- sense

restrict. enzyme: XbaI
 promotor: T7

Additional comments:

- Plasmid contains complete ORF of XPax-2a (1)

pBluescript® II SK (+) phagemid

The pBluescript® II SK (+/-) phagemid is a 2961-bp phagemid derived from pUC19. The SK designation indicates the polylinker is oriented such that *lacZ* transcription proceeds from *Sac* I to *Kpn* I.

fl (+) origin: (3-439 bp) fl filamentous phage origin of replication allowing recovery of the sense strand of the *lacZ* gene when a host strain containing the pBluescript II phagemid is co-infected with helper phage.

fl (-) origin: (3-439 bp) fl filamentous phage origin of replication allowing recovery of the antisense strand of the *lacZ* gene when a host strain containing the pBluescript II phagemid is co-infected with helper phage.

ColE1 origin: (1032-1972 bp) Plasmid origin of replication used in the absence of helper phage.

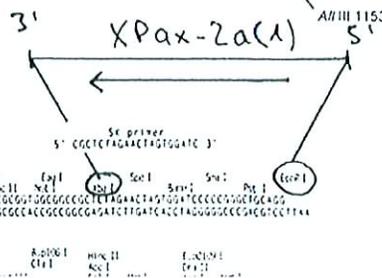
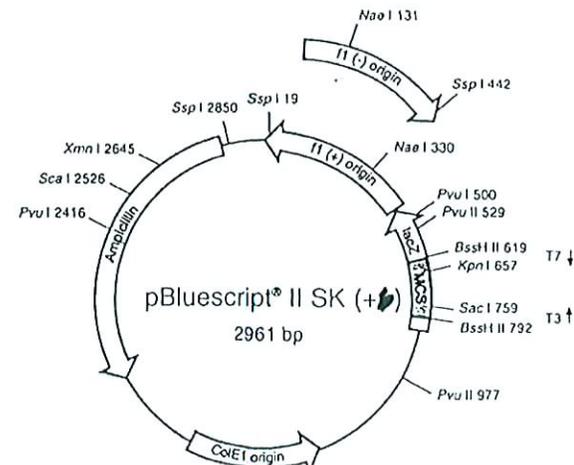
***lacZ* gene:** (*lac* promoter: 816-938 bp) This portion of the *lacZ* gene provides α-complementation for blue/white color selection of recombinant phagemids. An inducible *lac* promoter upstream from the *lacZ* gene permits fusion protein expression with the β-galactosidase gene product.

MCS: (657-739 bp) Multiple cloning site flanked by T3 and T7 RNA promoters (please see the polylinker sequence below)

Ampicillin: (1975-2832 bp) Ampicillin-resistance gene (*Amp^r*) for antibiotic selection of the phagemid vector.

Please Note: The upper strand is designated the (+) strand and the lower strand is designated the (-) strand.

GenBank # X52324 [SK(+)] & # X52330 [SK(-)]



pCS2+ Xror2

from: Masanori Taira
Ref: Hikasa et al. 2002

Insert: Xror2 ORF

