Modification Form for Permit BIO-UWO-0019

Permit Holder: Shun-Cheng (Shawn) Li

Approved Personnel
(Please stroke out any personnel to be removed)

~~Karen Kennedy~~ (gone)
Shelly Sandiford
~~Thamara Dayarathna~~ (gone)
~~Zezhou Wang~~ (gone)
Chengjun Li
Ran Wei
~~Marek Galka~~ (gone)
Courtney Voss
Gurpreet Dhami
Xuan Cao

Additional Personnel
(Please list additional personnel here)

Huadong Liu
Wendy Zhu
Bin Liu
Xuguang Liu
Xing Li

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E-coli. DH5 alpha, BL21,	
Approved Primary and Established Cells	[Established] (Human): HEK293. (Other): P19, MDCK2	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid]: pET-28a, pGEX-4T3	
Approved Use of Animals	mice (C57/BL6 and Balb/C)	
Approved Biological Toxin(s)		cholera toxin

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder Qiyun Li (on behalf of Shawn Li)

Current Classification: 2 **Containment Level for Added Biohazards:**

Date of Last Biohazardous Agents Registry Form: Jan 24, 2011

Date of Last Modification (if applicable):

BioSafety Officer(s):

Chair, Biohazards Subcommittee: **Date:**

Passage of MCF-10A mammary epithelial cells in monolayer culture.

Introduction: This protocol describes the passage and maintenance of MCF-10A epithelial cells (Figure 1). MCF-10A cells are an immortalized, non-transformed epithelial cell line derived from human fibrocystic mammary tissue. These cells are defined as "normal" breast epithelial cells as they have a near diploid karyotype and are dependent on exogenous growth factors for proliferation. They also lack the ability to form tumors in nude mice and lack the ability to grow in anchorage independent assays[1]. MCF-10A cells are an excellent model system for understanding epithelial cell biology. When plated in a mixture of collagen and laminin they form 3D structures that resemble acini structures of the human breast [2,3].

Materials:

Growth Media
Resuspension Media
Sterile PBS
Trypsin:EDTA (0.05% Trypsin: 0.53mM EDTA, Invitrogen, Cat# 25300)

Methods:

1. Aspirate old media and rinse with 10.0 ml PBS
2. Aspirate PBS and add 2 ml of 1X trypsin
3. Aspirate the majority of the trypsin leaving a thin film on the plate
4. Incubate @ 37° for 15-25 min. Check cells after 10 min and give a gentle tap. Continue to monitor cells every few minutes. Be sure the cells are well trypsinized. Partial trypsinization will result in clonal selection of the cells.
5. Add 1.0ml of resupension media to the trypsinized cells. Resuspend the cells using a p1000 tip to generate a single cell suspension. Transfer cells to a 15 ml conical tube and rinse the plate with 2.0ml of resuspension media. Note: If working with multiple plates, it is important to process one plate at a time, as the cells will re-attach to the plate once serum is added.
6. Spin cells at 150g for 3 minutes
7. Aspirate the media and resuspend the cells in 1.0 ml growth media.
8. Plate cells at a 1:4 dilution (typically 2.0X10^6 cells/10 cm plate). Cells will become confluent in about 3-4 days, split cells on Day 4

Important Notes:

- We strongly recommend maintaining the cells in this growth media with all the components.
- Keep track of the passage number.
- It is advisable to passage cells every 4 days. Keep in mind that the more you mishandle the cells the likely you will see them misbehave in 3D assays. MCF-10A cells behave well in 3D until about passage 25 to 30.
- If you plan to generate engineered cell lines, it is suggested that you start with cells that are less than passage 15.

Freezing Media:
Growth media
10%Fetal Bovine Serum
5% DMSO

Media Components:
DMEM/F12: Invitrogen Catalog # 11965-118
EGF: Peprotech; Catalog # 100-15. Prepare 100µg/ml stock solution in sterile water.
Hydrocortizone: Sigma; Catalog # H-0888. Make stock solution in 95% ethanol and store at –20°C.
CholeraToxin: Sigma; Catalog # C-8052. Make stock solution in water and store at 4°C. Take extra precautions when preparing the stock solution. Remember, this 'is' cholera toxin! If ingested will cause severe diarrhea.
Insulin: Sigma, Catalog # I-1882. Stock solutions (10mg/ml) should be prepared in acidified-water (100µl or glacial acetic acid in 10 ml of sterile water)
Pen/Strep: Invitrogen Catalog # 15070 - 063
Horse serum: Invitrogen Catalog #. 16050-122

References:

1. Soule, H.D., et al., *Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10.* Cancer Res, 1990. **50**(18): p. 6075-86.
2. Muthuswamy, S.K., et al., *ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini.* Nat Cell Biol, 2001. **3**(9): p. 785-92.
3. Debnath, J., S.K. Muthuswamy, and J.S. Brugge, *Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures.* Methods, 2003. **30**(3): p. 256-68.

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Material Number	Description	Quantity		Regulatory Reason
C8052-.5MG	CHOLERA TOXIN FROM VIBRIO CHOL	1	EA	US DEPT OF COMMERCE LICENSEABLE ITEM

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Thank you for your recent order with Sigma-Aldrich Canada Ltd. The product you have ordered is regulated by the U.S. Department of Commerce. Please note that if the above product is also regulated for import under the Human Pathogen Importation Regulations, and/or the Canadian Food Inspection Agency, you will be forwarded additional information/form (s) separately.

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Customer Number: 4965453 | Sales Order Number: 3006542489

Material Number: C8052-.5MG Description: Cholera Toxin from Vibrio Chol

TYPE OR PRINT CLEARLY

Part A: Institution
Institution Name: The University of Western Ontario

Department Name: Biochemistry

Street Address: 1400 Western Road, SDRI Rm112

City, Province: London, Ontario

Country: Canada Postal Code N6G 2V4

Telephone # 5196612111 ext 85648 Fax # 519-661-3175

Website address: www.uwo.ca

Part B: End User Information
End User: Xuan Cao
Full name please, no initials)

Email Address: xcao25@uwo.ca

Past Research/Educational Background Information:
2008.10 – Present : post doctoral fellow in Uwo. Canada.
2005.9 – 2008.7 : phD student in Wuhan University. China
2003.9 – 2005.7 : master student in Wuhan University, China.

Detailed end usage: Please be specific and define any technical terms or jargon used. General statement such as, For research use only are unacceptable. Provide literature references where possible.

Cholera Toxin is the component of MCF10A cells culture media. As for the literature reference.

you can access the following website:
http://muthuswamylab.cshl.edu/ml_protocols.html

Part C: Additional Parties Involved
Name/Address of all intermediates involved in this transaction (freight forwarders, brokers, distributors, dealers)

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Declaration

I agree 1) not to use this product in chemical or biological weapons manufacture or applications. 2) to use this product only for the application stated above.3)

Printed Name: Chengjun Li

Signature: Chengjun Li

Title/Position: Technician

Date: July 29, 2011

Material Safety Data Sheet

Version 4.2
Revision Date 11/05/2010
Print Date 08/09/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Cholera Toxin Vibrio cholerae
Product Number	:	C8052
Brand	:	Sigma
Product Use	:	For laboratory research purposes.

Supplier	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA	Manufacturer	: Sigma-Aldrich Corporation 3050 Spruce St. St. Louis, Missouri 63103 USA
Telephone	:	+1 9058299500		
Fax	:	+1 9058299292		
Emergency Phone # (For both supplier and manufacturer)	:	1-800-424-9300		
Preparation Information	:	Sigma-Aldrich Corporation Product Safety - Americas Region 1-800-521-8956		

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Bowel

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate eye irritant

GHS Classification

Acute toxicity, Oral (Category 5)
Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific target organ toxicity - single exposure (Category 3)

GHS Label elements, including precautionary statements

Pictogram

Signal word: Warning

Hazard statement(s)

H303	May be harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HMIS Classification

Health hazard: 2

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

Potential Health Effects

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

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : Cholera enterotoxin
Cholergen

CAS-No.	EC-No.	Index-No.	Concentration
Tris (hydroxymethyl) aminomethane			
77-86-1	201-064-4	-	>= 5.82 - <= 5.94 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	>= 31.3 - <= 31.9 %
Sodium chloride			
7647-14-5	231-598-3	-	>= 57.6 - <= 58.8 %
Exotoxin, vibrio cholerae			
9012-63-9	-	-	>= 0.5 - <= 2.5 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	>= 0.96 - <= 0.98 %

4. FIRST AID MEASURES

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

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

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

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

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

5. FIRE-FIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

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

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

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

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

Environmental precautions
Do not let product enter drains.

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

7. HANDLING AND STORAGE

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

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

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
For nuisance exposures use type P95 (US) or type P1 (EU EN 143) particle respirator.For higher level protection use type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

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

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

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

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

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

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	solid
Colour	no data available

Safety data

pH	no data available
Melting/freezing	no data available

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

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
Dimethyl sulfate, Acid chlorides, Halogenated hydrocarbon, Metals, Acids

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

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

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

Aspiration hazard
no data available

Potential health effects

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

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Exotoxin, vibrio cholerae	9012-63-9

WHMIS Classification

D2B	Toxic Material Causing Other Toxic Effects	Moderate skin irritant Moderate eye irritant

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

16. OTHER INFORMATION

Further information

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