

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

Approved Personnel

(Please stroke out any personnel to be removed)

Jacob Turowec
 Laszlo Gyenis
 Nicole St-Denis
 Greg Vilk
 Jennifer Raaf
 Dana Onica
 Michelle Gabriel
 Kathryn Garside

Additional Personnel

(Please list additional personnel here)

Jan Kreutzer
 Brendan Innis
 Mohammed Al-Sorkhy

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, XL1 Blue, BL21, S. Cerevisia

--

Approved Primary and Established Cells

Human (established), U2OS, HeLa, PANC-1
 Rodent (established), various fibroblast lines,
 Non-human primate (established), Cos7

--

Approved Use of Human Source Material

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--

Approved Genetic Modifications (Plasmids/Vectors)

SV 40 Large T antigen, Cos7 cells, CK2 protein, pCDNA3-Casp8, pET15b-Casp8 delta DED, pET15b-Casp8 delta DED C3600A, pCDNA3-Casp8 C360A, HDAC4 Flag, pmAmetrine-DEVD-IId Tomato, pJ3H-

pCDNA4-Chk1-Flag

Approved Use of Animals

--

--

Approved Biological Toxin(s)

Okadaic acid

--

* 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: 

Current Classification: 2 Containment Level for Added Biohazards: 1

Date of Last Biohazardous Agents Registry Form: Dec 14, 2007

Date of Last Modification (if applicable): Sep 17, 2010

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ Date: _____



MATERIAL SAFETY DATA SHEET
E. coli bacteria with plasmids

Last Updated: 03/10/2006
Version 1.02

SECTION 1: PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: E. coli bacteria with plasmids
COMPANY: Addgene
STREET ADDRESS: One Kendall Square, Building 600, 3rd Floor
CITY, STATE, ZIP, COUNTRY: Cambridge, MA 02139 US
TELEPHONE: (617) 225-9000
FAX: (888) 734-0533
PRODUCT USE: For laboratory research use only.

SECTION 2: COMPOSITION/INFORMATION ON INGREDIENTS

SUBSTANCE: E. coli bacteria
CAS NO. None
SARA 313 REPORTABLE No

SECTION 3: HAZARDS IDENTIFICATION

ROUTES OF ENTRY: Skin or eye contact
POTENTIAL HEALTH EFFECTS
EYES: May cause irritation of the eye
SKIN: May cause irritation of the skin.
INGESTION: May cause nausea or vomiting.
INHALATION: No toxicity expected from inhalation.
CARCINOGENICITY Not listed by OSHA, IARC, or NTP

SECTION 4: FIRST AID MEASURES

EYES: Rinse opened eyes for at least 15 minutes. If eyes become irritated, get medical attention.
SKIN: Wash skin with soap and water.
INGESTION: Rinse mouth. If irritation or symptoms occur, get medical attention.
INHALATION: Find fresh air. If symptoms occur, get medical attention.

SECTION 5: FIRE-FIGHTING MEASURES

EXTINGUISHING MEDIA: Water, carbon dioxide, dry chemical powder, or foam
SPECIAL PROCEDURES: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

SECTION 6: ACCIDENTAL RELEASE MEASURES

ENVIRONMENTAL PRECAUTIONS: Keep out of sewers and waterways.

CLEAN-UP METHODS: Clean spills with chlorine bleach or 70% ethanol. Autoclave materials and dispose of waste in accordance with federal, state, and local safety regulations.

SECTION 7: HANDLING AND STORAGE

HANDLING: Avoid inhalation and contact with eyes, skin, and clothing.

STORAGE: Store at 4°C. Create frozen stocks for long-term storage.

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS: Safety shower and eye bath

VENTILATION : General ventilation

RESPIRATORY PROTECTION: Not required

EYE PROTECTION: Safety goggles

SKIN PROTECTION: Protective gloves

WORK HYGIENIC PRACTICES: Use safe laboratory practices. Handle as biohazard material.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

FORM: Bacteria supplied in glass vial with LB agar.

ODOR: Odorless

COLOR: Colorless

SECTION 10: STABILITY AND REACTIVITY

STABILITY: Stable

CONDITIONS TO AVOID: None known

MATERIALS TO AVOID: None known

**HAZARDOUS DECOMPOSITION
OR BY-PRODUCTS:** None known

HAZARDOUS POLYMERIZATION: None

SECTION 11: TOXICOLOGICAL INFORMATION

TOXICOLOGICAL INFORMATION: May be irritating to eyes and skin.

SECTION 12: ECOLOGICAL INFORMATION

ECOLOGICAL INFORMATION: No data.

SECTION 13: DISPOSAL CONSIDERATIONS

WASTE DISPOSAL METHOD: Observe all federal, state, and local laws and regulations.

RCRA HAZARD CLASS: Not applicable.

SECTION 14: TRANSPORT INFORMATION

U.S. DEPARTMENT OF TRANSPORTATION
HAZARD CLASS: Non-hazardous

WATER TRANSPORTATION
HAZARD CLASS: Non-hazardous

AIR TRANSPORTATION
HAZARD CLASS: Non-hazardous

SECTION 15: REGULATORY INFORMATION

OSHA REGULATORY STATUS
(29 CFR 1910.1200) Irritant

TSCA: No specific regulations

CERCLA: None

SARA TITLE III: None

SARA 311/312 HAZARD CATEGORIES: None

SARA 313 REPORTABLE INGREDIENTS: None

STATE REGULATIONS: N/A

SECTION 16: OTHER INFORMATION

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

The above information is correct to the best of our knowledge, but does not purport to be all inclusive and shall be used only as a guide. Addgene shall not be held liable for any damage resulting from handling or from contact with the above product.

The plasmid being added to our biohazard list, pcDNA4-Flag-Chk1, will be used for expression studies in mammalian cells. This will involve transfection of the plasmid into cells and isolation of the protein product for mass spectrometry and western blot analysis. The plasmid will also be subcloned into a bacterial expression vector for protein purification. The E.coli stock will be stored in our -80°C freezer, and purified plasmid will be stored at 4°C. All experimental materials will be disposed of with our biohazardous waste.

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(Please stroke out any personnel to be removed)

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(Please list additional personnel here)

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Write additional Biohazards for approval below. Give the full name - do not abbreviate.

Approved Microorganisms

E. coli, DH5 alpha, XL1 Blue, BL21, S. Cerevisea

Approved Primary and Established Cells

Human (established), U20S, HeLa, Rodent (established), various fibroblast lines, Non-human primate (established), Cos7

PANC-1

Approved Use of Human Source Material

Approved Genetic Modifications (Plasmids/Vectors)

SV 40 Large T antigen, Cos7 cells, CK2 protein, pCDNA3-Casp8, pET15b-Casp8 delta DED, pET15b-Casp8 delta DED C3600A, pCDNA3-Casp8 C360A, HOAC4 Flag, pmAmetrine-DEVD-td Tomato, pJ3H-

Approved Use of Animals

Approved Biological Toxin(s)

Okadaic acid

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Signature of Permit Holder: *David Atkinson*
Current Classification: 2 Containment Level for Added Biohazards: 1
Date of Last Biohazardous Agents Registry Form: Dec 14, 2007
Date of Last Modification (if applicable): Aug 13, 2010
BioSafety Officer(s): J Stanley Sept 16/10.
Chair, Biohazards Subcommittee: *Miller* Date: Sept 17/10

----- Original Message -----

Subject:Re: Fwd: update of Biosafety permit needed - Litchfield lab

Date:Tue, 07 Sep 2010 10:39:29 -0400

From:Laszlo Gyenis <lgyenis@uwo.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

CC:David Litchfield <litchfi@uwo.ca>

Hi Jennifer,

The PANC-1 cell will be used for biochemical studies. They will be transfected with plasmid DNA to overexpress proteins (e.g. CK2, EGFP) to monitor cell signaling event changes. We will do similar experiments that we do with the HeLa cells.

Please let me know if you would need more detailed information on the experiments.

Regards, Laszlo



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Product Description

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Cell Biology

ATCC® Number:	CRL-1469™	Order this Item	Price:	\$256.00
Designations:	PANC-1		Related Links	
Depositors:	M Lieber		▶	
Biosafety Level:	1		NCBI Entrez Search	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human)		Technical Support	
Morphology:	epithelial		Related Cell Culture Products	
Source:	Organ: pancreas Tissue: duct Disease: epithelioid carcinoma		Login Required	
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		▶	
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		Product Information Sheet	
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 11 D16S539: 11 D5S818: 11,13 D7S820: 8,10 TH01: 7,8 TPOX: 8,11 vWA: 15			
Cytogenetic Analysis:	Chromosome studies indicate a modal number of 63 with 3 distinct marker chromosomes and a small ring chromosome. This is a hypertriploid human cell line. The modal chromosome number was 61, occurring in 32% of cells. However, cells with 63 chromosomes also occurred at a high frequency (22%). The rate of cells with higher ploidies was 8.5%.			
Isoenzymes:	G6PD, B			
Age:	56 years			
Gender:	male			
Ethnicity:	Caucasian			
Comments:	Growth is inhibited by 1 unit/ml L-asparaginase The cells will grow in soft agar			

Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C Atmosphere: air, 95%, carbon dioxide (CO ₂), 5%
Subculturing:	Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended Medium Renewal: 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.53mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	culture medium 95%: DMSO, 5%
Doubling Time:	52 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2002 recommended serum: ATCC 30-2020
References:	22850: Lieber M, et al. Establishment of a continuous tumor-cell line (panc-1) from a human carcinoma of the exocrine pancreas. <i>Int. J. Cancer</i> 15: 741-747, 1975. PubMed: 1140870 22859: Wu MC, et al. Mechanism of sensitivity of cultured pancreatic carcinoma to asparaginase. <i>Int. J. Cancer</i> 22: 728-733, 1978. PubMed: 363626 23079: Lan MS, et al. Polypeptide core of a human pancreatic tumor mucin antigen. <i>Cancer Res.</i> 50: 2997-3001, 1990. PubMed: 2334903

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- Greg Vilk

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pET23b-Casp3-C186A-HIS
pCDNA3-Casp7-FLAG
pCDNA3-Casp7-C180A-FLAG
pET23b-Casp7-HIS
pET23b-Casp7-C186A-HIS
pET23b-Casp9-HIS

Approved Use of Animals

Approved Biological Toxin(s)

Okadaic acid

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Signature of Permit Holder: 

Current Classification: 2 Containment Level for Added Biohazards: _____

Date of Last Biohazardous Agents Registry Form: Dec 14, 2007

Date of Last Modification (if applicable): Mar 18, 2009

BioSafety Officer(s): J Stanley Aug 13, 2010

Chair, Biohazards Subcommittee: Susan Koval Date: August 13, 2010

10/07/13 Statement of Intent for Modification form BIO-UWO-0069

The requested pET23b plasmids will be used for transformation into BL21 *E. coli* followed by protein isolation. The isolated caspases will be used in enzymatic reactions. The pCDNA3 plasmids will be used for transfection of cultured mammalian cells for the purpose of over-expressing caspase-7. Isolated lysates will be used in various molecular biology applications (western blots, etc.). All information pertaining to these plasmids as provided by Addgene are attached.

Plasmid 11826: pET23b-Casp7 C186A-His

Gene/insert name:	Caspase 7 catalytic mutant
Alternative names:	Caspase 7
Insert size (bp):	1053
Gene/insert aliases:	CASP7, MCH3, CMH-1, ICE-LAP3
Species of gene(s):	H. sapiens (human)
Relevant mutations/deletions:	C186A
Fusion proteins or tags:	6xHis
Terminal:	C terminal on insert
Vector backbone:	pET-23 b (Search Vector Database)
Type of vector:	Bacterial expression
Backbone size (bp):	3666
Cloning site 5':	XhoI
Site destroyed during cloning:	Unknown
Cloning site 3':	NdeI
Site destroyed during cloning:	Unknown
5' Sequencing primer:	T7 terminal primer (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/11826
Plasmid Provided In:	DH5a
Principal Investigator:	Guy Salvesen

Article: [Target protease specificity of the viral serpin CrmA. Analysis of five caspases.](#) Zhou Q et al. (J Biol Chem. 1997 Mar 21. 272(12):7797-800. [Pubmed](#))

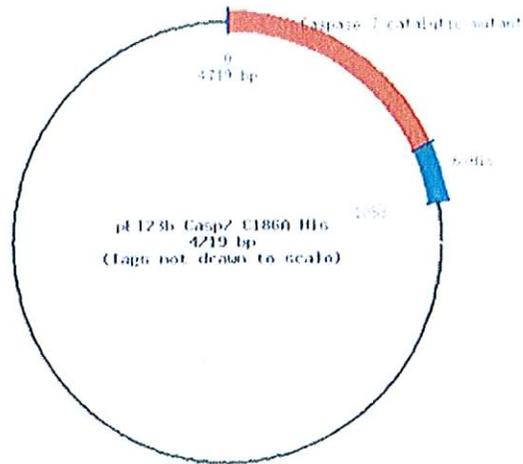
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11826" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11826 for updated plasmid information and related links.

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Plasmid 11825: pET23b-Casp7-His

Gene/insert name:	Caspase 7
Insert size (bp):	1000
Gene/insert aliases:	CASP7, MCH3, CMH-1, ICE-LAP3
Species of gene(s):	H. sapiens (human)
Fusion proteins or tags:	6xHis
Terminal:	C terminal on insert
Vector backbone:	pET-23 b (Search Vector Database)
Type of vector:	Bacterial expression
Backbone size (bp):	3666
Cloning site 5':	XhoI
Site destroyed during cloning:	Unknown
Cloning site 3':	NdeI
Site destroyed during cloning:	Unknown
5' Sequencing primer:	T7 terminal primer (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/11825
Plasmid Provided In:	DH5a
Principal Investigator:	Guy Salvesen

Article: [Target protease specificity of the viral serpin CrmA. Analysis of five caspases.](#) Zhou Q et al. (J Biol Chem. 1997 Mar 21. 272(12):7797-800. [Pubmed](#))

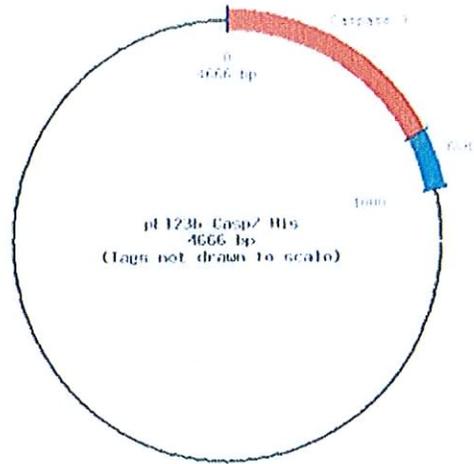
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

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Please check www.addgene.org/11825 for updated plasmid information and related links.

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Plasmid 11822: pET23b-Casp3 C163A-His

Gene/insert name: Caspase 3 catalytic mutant
Alternative names: Caspase 3
Insert size (bp): 999
Gene/insert aliases: CASP3, CPP32, SCA-1, CPP32B
Species of gene(s): H. sapiens (human)
Relevant mutations/deletions: C163A
Fusion proteins or tags: 6xHis
Terminal: C terminal on insert
Vector backbone: pET-23 b
([Search Vector Database](#))
Type of vector: Bacterial expression
Backbone size (bp): 3666
Cloning site 5': XhoI
Site destroyed during cloning: Unknown
Cloning site 3': NdeI
Site destroyed during cloning: Unknown
5' Sequencing primer: T7 terminal primer ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Sequence: Visit www.addgene.org/11822
Plasmid Provided In: DH5a
Principal Investigator: Guy Salvesen

Article: [Target protease specificity of the viral serpin CrmA. Analysis of five caspases](#), Zhou Q et al. (J Biol Chem. 1997 Mar 21. 272(12):7797-800. [Pubmed](#))

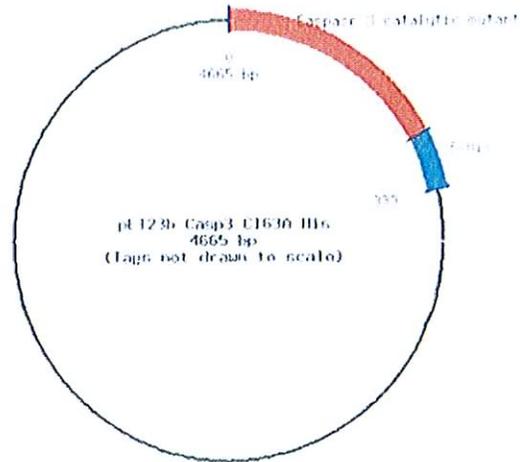
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11822" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11822 for updated plasmid information and related links.

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Plasmid 11829: pET23b-Casp9-His

Gene/insert name: Caspase 9
Insert size (bp): 1373
Gene/insert aliases: CASP9, MCH6, APAF3, APAF-3, ICE-LAP6, CASPASE-9c
Species of gene(s): H. sapiens (human)
Fusion proteins or tags: 6xHis
Terminal: C terminal on insert
Vector backbone: pET-23 b
([Search Vector Database](#))
Type of vector: Bacterial expression
Backbone size (bp): 3666
Cloning site 5': Don't Know
Site destroyed during cloning: Unknown
Cloning site 3': Don't Know
Site destroyed during cloning: Unknown
5' Sequencing primer: T7 terminal primer ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Sequence: Visit www.addgene.org/11829
Plasmid Provided In: DH5a
Principal Investigator: Guy Salvesen

Article: [Caspase-9 can be activated without proteolytic processing](#), Stennicke HR et al. (J Biol Chem. 1999 Mar 26; 274(13):8359-62. [Pubmed](#))

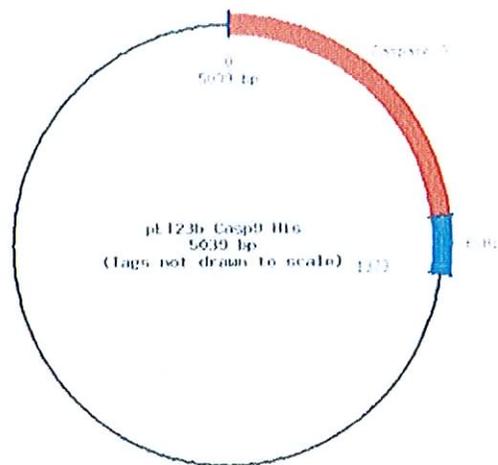
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Plasmid 11816: pcDNA3-Casp7 C186A-Flag

Gene/insert name: Caspase 7 catalytic mutant
Alternative names: Caspase 7
Insert size (bp): 972
Gene/insert aliases: CASP7, MCH3, CMH-1, ICE-LAP3
Species of gene(s): H. sapiens (human)
Relevant mutations/deletions: C186A
Fusion proteins or tags: Flag
Terminal: C terminal on insert
Vector backbone: pcDNA3
([Search Vector Database](#))
Backbone manufacturer: Invitrogen
Type of vector: Mammalian expression
Backbone size (bp): 5446
Cloning site 5': KpnI
Site destroyed during cloning: Unknown
Cloning site 3': XhoI
Site destroyed during cloning: Unknown
5' Sequencing primer: T7 ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Selectable markers: Neomycin
Sequence: Visit www.addgene.org/11816
Plasmid Provided In: DH5a
Principal Investigator: Guy Salvesen

Article: [Biochemical characteristics of caspases-3, -6, -7, and -8](#). Stennicke HR et al. (J Biol Chem. 1997 Oct 10. 272(41):25719-23. [Pubmed](#))

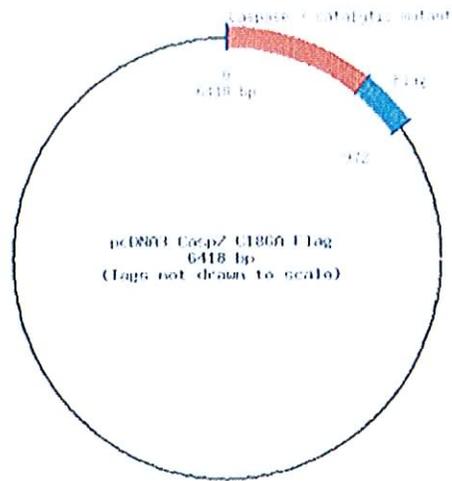
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Plasmid 11815: pcDNA3-Casp7-Flag

Gene/insert name: Caspase 7
Insert size (bp): 1000
Gene/insert aliases: CASP7, MCH3, CMH-1, ICE-LAP3
Species of gene(s): H. sapiens (human)
Fusion proteins or tags: Flag
Terminal: C terminal on insert
Vector backbone: pcDNA3
([Search Vector Database](#))
Backbone manufacturer: Invitrogen
Type of vector: Mammalian expression
Backbone size (bp): 5446
Cloning site 5': KpnI
Site destroyed during cloning: Unknown
Cloning site 3': XhoI
Site destroyed during cloning: Unknown
5' Sequencing primer: T7 ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: Yes
Selectable markers: Neomycin
Sequence: Visit www.addgene.org/11815
Plasmid Provided In: DH5a
Principal Investigator: Guy Salvesen

Article: [Biochemical characteristics of caspases-3, -6, -7, and -8](#). Stennicke HR et al. (J Biol Chem. 1997 Oct 10; 272(41):25719-23. [Pubmed](#))

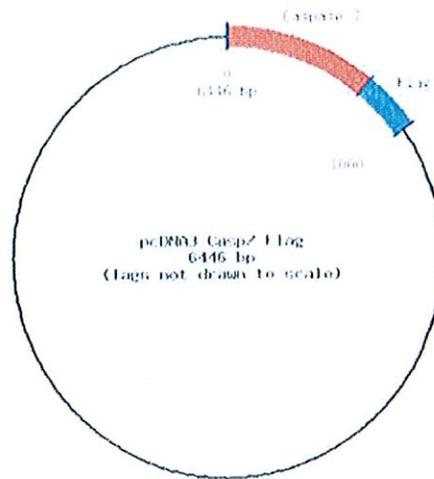
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 11815" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/11815 for updated plasmid information and related links.

Page 1 of 2 - Date: 07/13/2010

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Please check www.addgene.org/11815 for updated plasmid information and related links.

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Information on this datasheet is provided pursuant to Addgene's Terms of Use at www.addgene.org.

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

Approved Personnel

(Please stroke out any personnel to be removed)

Kathryn Garside
 Deborah Ng
 Jacob Turowec
 Laszlo Gyenis
 Nicole St-Denis
 Melanie Bailoy
 Erin Parker
 Ashley French

Additional Personnel

(Please list additional personnel here)

Michelle Gabriel
 Dana Onica
 Greg Vilk
 Jennifer Raaf

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli, DH5 alpha, XL1 Blue, BL21, S. Cerevisiae	
Approved Cells	Human (established), U2OS, HeLa, Rodent (established), various fibroblast lines, Non-human primate (established), Cos7	
Approved Use of Human Source Material		
Approved GMO	SV 40 Large T antigen, Cos7 cells, CK2 protein, pCDNA3-Casp8, pET15b-Casp8 delta DED, pET15b-Casp8 delta DED C3600A, pCDNA3-Casp8 C360A, HDAC4 Flag, pmAmetrine-DEVD-IId Tomato	pJ3H-Mst2 K56R pJ3I-I-Mst1
Approved use of Animals		
Approved Toxin(s)	Okadaic acid	

MSDS and Description

There is no MSDS that I am aware of for the requested products. The pJ3H-MST2 K56R and pJ3H-MST1 DNA plasmids are shipped as bacterial stabs in the E. coli strain DH5 α (an approved microorganism on our permit). These E. coli will be grown and the plasmid DNA isolated for use in transfection of approved mammalian cell lines. Cell lysates will be analyzed using various biochemical methods.


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Price: \$65.00

Plasmid 12203: pJ3H-Mst1

Gene/insert name: Mst1
 Insert size (bp): 1600
 Gene/insert aliases: MST1, MSP, I-GFL, NF15S2, ID3F15S2, DNF15S2, STK4, KRS2, MST1, YSK3, DKFZp686A2068
 Species of gene(s): H. sapiens (human)
 Fusion proteins or tags: HA
 Terminal: N terminal on backbone
 Vector backbone: pJ3H
[\(Search Vector Database\)](#)
 Type of vector: Mammalian expression
 Backbone size (bp): 3500
 Cloning site 5': BamHI
 Site destroyed during cloning: No
 Cloning site 3': EcoRI
 Site destroyed during cloning: No
 5' Sequencing primer: SV40pro-F [\(List of Sequencing Primers\)](#)
 Bacteria resistance: Ampicillin
 High or low copy: High Copy
 Grow in standard E. coli @ 37C: Yes
 Sequence: [View sequence](#)
 Plasmid Provided in: DH5a
 Principal Investigator: Jonathan Chernoff
 Terms and Licenses: [MTA](#)

Plasmid Links
Sequence
Reviews (0)
Related Plasmids
From this article
STK4 plasmids
MST1 plasmids
Jonathan Chernoff Lab Plasmids
Other Links
NCBI: STK4
NCBI: MST1
STK4 antibodies

This is commonly requested with
pJ3M-Mst1 K59R
pJ3M-Mst2
pJ3H-Mst2 K56R

Plasmid Cart

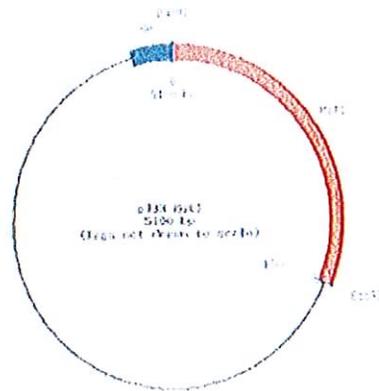
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Article: [The Ste20-like protein kinase, MstI, dimerizes and contains an inhibitory domain.](#)
Creasy CL et al. (J Biol Chem. 1996 Aug 30; 271(35):21049-53. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 12203" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.



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Price \$65.00

Plasmid 12206: pJ3H-Mst2 K56R

Gene/insert name: Mst2
 Insert size (bp): 2000
 Gene/insert aliases: STK3, KRS1, MST2, FLJ90748
 Species of gene(s): H. sapiens (human)
 Relevant mutations/deletions: Catalytically inactive: K56R
 Fusion proteins or tags: HA
 Terminal: N terminal on backbone
 Vector backbone: pJ3H
 (Search Vector Database)
 Type of vector: Mammalian expression
 Backbone size (bp): 3500
 Cloning site 5': BamI II
 Site destroyed during cloning: No
 Cloning site 3': EcoRI
 Site destroyed during cloning: No
 5' Sequencing primer: SV40pro-F (List of Sequencing Primers)
 Bacteria resistance: Ampicillin
 High or low copy: High Copy
 Grow in standard E. coli @ 37C: Yes
 Sequence: [View sequence](#)
 Plasmid Provided In: DH5a
 Principal Investigator: Jonathan Chernoff
 Terms and Licenses: [MTA](#)

Plasmid Links
Sequence
Reviews (0)
Related Plasmids
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STK3 plasmids
Jonathan Chernoff Lab Plasmids
Other Links
NCBI: STK3

This is commonly requested with
pJ3M-Mst2
pJ3H-Mst1
pJ3M-Mst1 K59R

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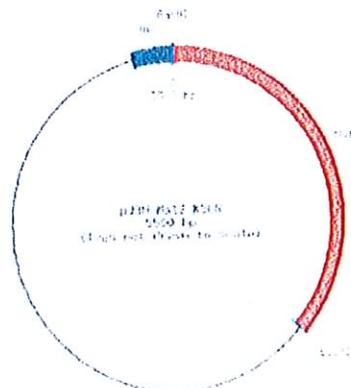
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[pJ3H-Mst2 K56R Plasmid 12206](#)

[pJ3H-Mst1 Plasmid 12203](#)

Addgene has sequenced a portion of this plasmid for verification. Click [here](#) for the sequencing result.

[Click on map to enlarge](#)



Article: [The Ste20-like protein kinase, Mst1, dimerizes and contains an inhibitory domain](#)
Creasy CL et al. *J Biol Chem*. 1996 Aug 30; 271(35):21049-53. [PubMed](#)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 12206" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Modification Form for Permit BIO-UWO-0069

Permit Holder: *David Birchfield*

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	<i>E. coli, DH5 alpha, XL1 Blue EL21, S. Cerevisiae</i>	
Approved Cells	<i>Human (established), U2OS, HeLa, Rodent (established), various fibroblast lines, Non-human primate (established), Cos7</i>	
Approved Use of Human Source Material		
Approved GMO	<i>SV 40 Large T antigen, Cos7 cells, CK2 protein, pCDNA3-Casp8, pET15b-Casp8 della DED, pET15b-Casp8 della DED C3600A, pCDNA3-Casp8 C360A, HDAC4 Flag</i>	<i>proAnelone-DEVD-tdTomato</i>
Approved use of Animals		
Approved Toxin(s)	<i>Okadaic acid</i>	

* 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 USED.

Classification: *2*

Date of last Biohazardous Agents Registry Form: *Dec 14, 2007*

Signature of Permit Holder: *David Birchfield*

BioSafety Officers: *Alan... Jan 30/09*

Chair, Biohazards Subcommittee: *Leslie K... 2009*

Modification Form for Permit BIO-UW-0-0069
Permit Holder: David Birchfield

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

- Elizabeth Roach -
- Jacob Turowes
- Laszlo Gyenis
- Nicci St-Denis
- ~~Kelly-Gundam~~
- Melanie Bailey
- Erin Parker
- Ashley French
- ~~James-Duncan~~
- ~~Rich Derksen~~

Additional Personnel
 (Please list additional personnel here)

- Greg Vilk
- Deborah Yuen Ling Ng
- Kathryn Garside

* 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 USED

Classification: 2

Date of last Biohazardous Agents Registry Form: Dec 14, 2007

Signature of Permit Holder: *David Birchfield*

BioSafety Officer(s): *M. Kuntley Jan 30/09*

Chair, Biohazards Subcommittee: *Christie K. ...*

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Hutchfield

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below, ^s
Approved Microorganisms	E. coli, DH5 alpha, Xt-1 Blue, BL21, S. cerevisiae	
Approved Cells	Human (established), U2OS, HeLa, Rodent (established), various fibroblast lines, Non human primate (established), Cos7	
Approved Use of Human Source Material		
Approved GMO	SV 40 Large T antigen, Cos7 cells, GK2 protein	<p>PCDNA3 - Casp8 pET15b - Casp8 delta DEE pET15b - Casp8 delta DEE C360A pCDNA3 - Casp8 C360A HDAC4 Flag</p>
Approved use of Animals		
Approved Toxin(s)	Okadaic acid	

* 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 USED.

Date of last Biohazardous Agents Registry Form: Dec 14, 2007

Signature of Permit Holder:

David Hutchfield

BioSafety Officer:

V. Stanley

Oct 23 / 08

Chair, Biohazards Subcommittee:

E. M. Kilder

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Ditchfield

Approved Personnel

(Please stroke out any personnel to be removed)

Elizabeth Roach
Jacob Furwec
Laszlo Gyenis
Nicole St-Denis
Kelly Duncan
Melanie Bailey
Elin Parker
Ashley French
James Duncan
Rich Derkson

Additional Personnel

(Please list additional personnel here)

- * 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 USED

Date of last Biohazardous Agents Registry Form Dec 14, 2007

Signature of Permit Holder:

David Ditchfield

BioSafety Officer(s):

V. Stanley

Chair, Biohazards Subcommittee:

Modification Form for Permit BIO-UWO-0069
Permit Holder: David Litchfield

Intended use for pCDNA3-casp 8, pCDNA3-casp 8 C360A and HDAC4 Flag:

These three plasmid constructs will be transfected into established human cell lines. Their regulation (ie phosphorylation, proteolysis) will be studied using *in vitro* and *in vivo* molecular techniques.

Intended use for pET15b-Casp 8 delta DED, pET15b-Casp 8 delta DED C360A

These plasmids will be electroporated into BL21 E. coli cells in order to produce purified caspase 8 protein. The purified caspase 8 protein will be used in *in vitro* caspase assays and as a substrate in phosphorylation assays.



B10-UWO-0069

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA)

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr. David Litchfield

SIGNATURE *David Litchfield*

DEPARTMENT Biochemistry

ADDRESS The University of Western Ontario
Medical Science Building
Rooms 359, 355 and 380 (labs) Room 350 (office)
London ON N6A 5C1

PHONE NUMBER 519-661-2111 ext 86849 (lab) 84186 (office)

EMAIL litchfi@uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 359/355/380
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Roberts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):

NCIC - Regulation and Role of CK2 during cell cycle progression
CIHR - Signaling pathways controlling proliferation and survival
OCRN - Rational design of novel modulators of cell proliferation

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NCIC OCRN CIHR

Name(s) of all personnel working under Principal Investigator's supervision in this location:
Paul Parker (Grad Student)
Melanie Baines (Grad Student)
Paul Thompson (Postdoctoral Fellow)

DATE OF COMPLETION SEP 18 2007 BY DAVID LITCHFIELD FOR UWO (PRINT NAME OF PRINCIPAL INVESTIGATOR)
SIGNATURE OF PRINCIPAL INVESTIGATOR

- iv) Ashley French (Research Associate)
- v) James Duncan (Grad Student)
- vi) Nicole St-Denis (Grad Student)
- vii) Laszlo Gyenis (Postdoctoral Fellow)
- viii) Jacob Turowec (Grad Student)
- ix) Rich Derksen (Research Associate)
- x) Elizabeth Roach (4th year student)

1.0 Microorganisms

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

1.2 Please complete the table below

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
E. Coli (DH5a)	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	10 L
E. Coli (XL1 Blue)	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	2 L
E. Coli (BL21)	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	10 L
S. Cerevisia	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	2 L

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required.

1 2 3

1.4 Source of microorganism(s) or biological agent(s)? Invitrogen, collaborative labs

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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	
Rodent	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	
Non-human primate	Yes <input type="checkbox"/> No <input checked="" type="checkbox"/>	
Other (specify):		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source

4.7 Will virus be infectious to humans or animals
via _____

YES: _____ NO: _____

4.8 Will this be expected to increase the Containment Level required

YES _____ X NO

5.0 Human Gene Therapy Trials

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

YES _____ X NO

5.2 Will virus be able to replicate in the host?

YES: _____ NO: _____

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained?

YES _____ NO: _____

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals?
NO
If no, please proceed to section 7.0

YES: _____ X

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 If using murine cell lines, have they been tested for murine pathogens? YES _____ NO: _____

7.0 Use of Animal species with Zoonotic Hazards

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

- ◆ Pound source dogs YES: _____ X NO
- ◆ Pound source cats YES: _____ X NO
- ◆ Sheep or goats YES: _____ X NO
- ◆ Non-Human Primates YES: _____ X NO If YES specify species _____
- ◆ Wild caught animals YES: _____ X NO If YES specify species _____
colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin _____, Okadaic acid (phosphatase inhibitor) _____

8.3 What is the LD₅₀ (specify species) of the toxin _____, 5628 mg/kg _____

9.0 Import Requirements

9.1 Will the agent be imported? YES: NO
If no, please proceed to Section 10.0
If yes, country of origin _____

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

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

9.4 Has the import permit been sent to OHS? YES: NO
If yes, Permit # _____

10.0 Training Requirements for Personnel named on Form

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

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS

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

SIGNATURE *David Fitzpatrick*

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 (2) 3

11.2 Has the facility been certified by OHS for this level of containment? YES NO

11.3 If yes, please give the date and permit number June 20, 2006 BIO-UWO-0069
to be reinspected Dec 2007
(new location)

12.0 Approvals

IUWO Biohazard Subcommittee

Signature *G.M. Kildner* Date 14 Dec '07

Safety Officer for institution where experiments will take place

Signature *[Signature]* Date Dec 14, 2007

Safety Officer for the city of Western Ontario (if different than above)

Signature _____ Date _____