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 Bailey~~

~~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. Cerevisea	
Approved Cells	Human (established), U20S, 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-td Tomato	pJ3H-Mst2 K56R pJ3H-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 DH5α (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.

Plasmid 12203: pJ3H-Mst1

Gene/insert name:	Mst1
Insert size (bp):	1600
Gene/insert aliases:	MST1, MSP, HGFL, NF15S2, D3F15S2, 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

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

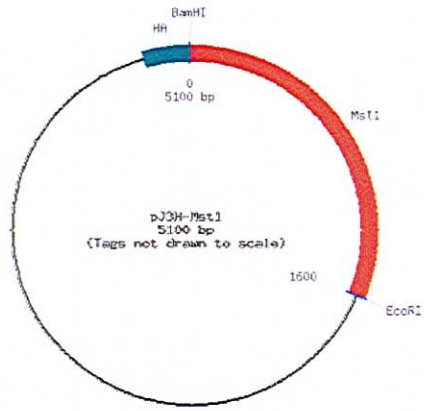
Click on map to enlarge

Add to Cart

Price: $65.00

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



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 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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Browse > Jonathan Chernoff > Creasy et al > pJ3H-Mst2 K56R

Print Friendly Email

Add to Cart

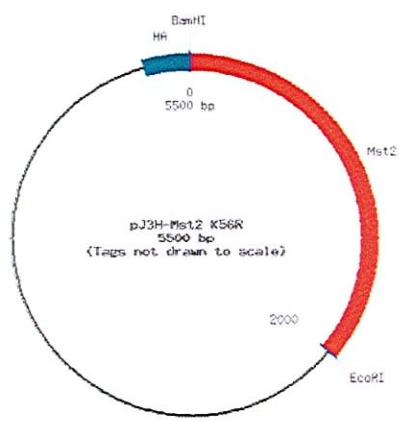
Price: $65.00

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':	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

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge



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

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

Plasmid Cart

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Recently Viewed

pJ3H-Mst2 K56R Plasmid 12206

pJ3H-Mst1 Plasmid 12203

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)

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Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

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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-tdTomato
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.*

Classification: 2

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

Signature of Permit Holder: David Litchfield

BioSafety Officer(s): A Hanley Jan 30/09

Chair, Biohazards Subcommittee: G M Kidder

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

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

~~Elizabeth Roach~~

Jacob Turowec

Laszlo Gyenis

Nicole St-Denis

~~Kelly Duncan~~

Melanie Bailey

Erin Parker

Ashley French

~~James Duncan~~

~~Rich Derkson~~

Additional Personnel
(Please list additional personnel here)

Greg Vilk

Deborah Yuen Ling Ng

Kathryn Garside

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Classification: 2

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

Signature of Permit Holder: David Litchfield

BioSafety Officer(s): JL Stanley Jan 30/09

Chair,Biohazards Subcommittee: GM Calder

We will be transfecting this plasmid into mammalian cells. The plasmid encodes a FRET molecule that can be used to monitor, via confocal microscopy, the activation of caspases - a group of proteolytic enzymes responsible for carrying out programmed cell death.

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

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Approved GMO	SV 40 Large T antigen, Cos7 cells, CK2 protein	pCDNA3 - Casp8 pETISb - Casp8 delta DED pETISb - Casp8 delta DED C360A pCDNA3 - Casp8 C360A HDAC4 Flag
Approved use of Animals		
Approved Toxin(s)	Okadaic acid	

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** *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 Litchfield

BioSafety Officer(s): J Hanley Oct 27/08

Chair, Biohazards Subcommittee: E M. Kidder

Modification Form for Permit BIO-UWO-0069

Permit Holder: David Litchfield

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

Elizabeth Roach

Jacob Turowec

Laszlo Gyenis

Nicole St-Denis

Kelly Duncan

Melanie Bailey

Erin Parker

Ashley French

James Duncan

Rich Derkson

Additional Personnel
(Please list additional personnel here)

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** *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 Litchfield

BioSafety Officer(s): Utsumy

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.

BIO-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 _____

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 Robarts 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 A 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_____

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

i. Erin Parker (Grad Student)
ii. Melanie Bailey (Grad Student)
iii. Kelly Duncan (Postdoctoral Fellow)

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

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)? X 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 X No	Yes X No	Yes X No	10 L
E. Coli (XL1 Blue)	Yes X No	Yes X No	Yes X No	2 L
E. Coli (BL21)	Yes X No	Yes X No	Yes X No	10 L
S. Cerevisea	Yes X No	Yes X No	Yes X No	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 microrganism(s) or biological agent(s)? _Invitrogen, collaborative labs_

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (ie. 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 X No	
Rodent	Yes X No	
Non-human primate	Yes X No	
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

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

Human	X Yes ☐ No	U20S, HeLa, many other types	Clontech, ATCC, collaborative labs
Rodent	X Yes ☐ No	Various fibroblast lines	Clontech, ATCC, collaborative labs
Non-human primate	X Yes ☐ No	Cos7	ATCC
Other (specify)	No ☐ Yes ☐		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 (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 if the following will be used in the laboratory
- Human blood (whole) or other bodily fluids NO ☐ YES ☐ If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human organs (unpreserved) NO ☐ YES ☐ If YES, Specify_____
- Human tissues (unpreserved) NO ☐ YES ☐ If YES, Specify_____

3.3 Is human source known to be infected with and infectious agent YES NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

4.0 Genetically Modified Organisms and Cell lines

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

4.2 Will genetic sequences from the following be involved
- HIV YES ☐ X NO

if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens YES ☐ X NO

if YES specify_____
- Other human or animal pathogen and or their toxins YES ☐ X NO

if YES specify_____

4.3 Will intact genetic sequences be used from
- SV 40 Large T antigen X YES ☐ NO If YES specify__Cos7 cells_____
- Known oncogenes X YES ☐ NO If YES specify__CK2 protein_____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES X NO
If YES name virus_____

4.5 List specific vector(s) to be used:_Recombinant plasmids with CMV promoters (for example: pBI, pRc/CMV, pTRE, pEGFP, etc)_

4.6 Will virus be replication defective YES ☐ NO
n/a

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

4.7 Will virus be infectious to humans or animals YES NO
n/a

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? YES X NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? 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? YES X
NO
If no, please proceed to section 7.0

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 LD_{50} (specify species) of the toxin_____5628 mg/kg_____

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

9.0 Import Requirements

9.1 Will the agent be imported? YES : X 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 _____

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? X 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

UWO Biohazard Subcommittee

Signature _____ Date 14 Dec. '07

Safety Officer for institution where experiments will take place

Signature _____ Date Dec 14, 2007

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

Signature _____ Date _____