Modification Form for Permit BIO-RRI-0020

Permit Holder: Joaquin Madrenas

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

Thu Chau

Luan Chau

Samar Sayedyahossein

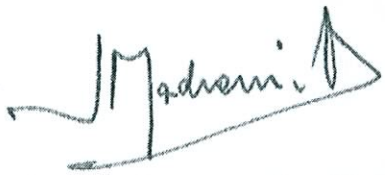
Darah Christie

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Superantigen	Commercially available S. aureus cell wall components
Approved Cells	Human (established) - E. 6.1 JurKats, HEK 293 E, Rodent (primary, established), [Primary] - (Human): blood, cell lines. (Rodent) - Mice	
Approved Use of Human Source Material	blood, h. CTLA4 pbig2i Plasmid. Peritoneal fluid, human SLP-2-gfp cDNA in pBIg2i plasmid.	Human TLR1, TLR2, and TLR6 cDNAs ready for expression in mammalian cells and signalling studies with S. aureus cell wall components.
Approved GMO	E. Coli DH5 Alpha. (Plasmid) - pBIG2i	
Approved use of Animals	2007-078-12 for MICE	
Approved Toxin(s)	superantigen, SEE (Staphylococcal Enterotoxin E)	

* *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.*

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: 

Classification: 2

Date of Last Biohazardous Agents Registry Form: Sep 23, 2009

Date of Last Modification (if applicable): Feb 4, 2010

BioSafety Officer(s): Ronald Rosenfeld March 04, 2010

Chair, Biohazards Subcommittee:

We are planning to study whether signalling from TLR2/1 and TLR2/6 complexes are different, both quantitatively and qualitatively, in response to ligands of TLR2 from the cell wall of the microbe Staphylococcus aureus. To do these experiments, we will transfect these constructs into fibroblasts and H293 cells, develop stable clones expressing these cDNAs, and examine their signalling response to
these ligands, following standard protocols already available in my laboratory.

Modification Form for Permit BIO-RRI-0020

Permit Holder: Joaquin Madrenas

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

~~Gaitlin Lemke~~

Thu Chau

~~Mark Kirchhof~~

Luan Chau

~~Brianne Davis~~

~~Sara Ramos~~

Samar Sayedyahossein

Darah Christie

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Superantigen	
Approved Cells	Human (established) - E. 6.1 JurKats, HEK 293 E, Rodent (primary, established), [Primary] - (Human): blood, cell lines. (Rodent) - Mice	
Approved Use of Human Source Material	blood, h. CTLA4 pbig2i Plasmid. Peritoneal fluid	human SLP-2-gfp cDNA in pBIG2i plasmid
Approved GMO	E. Coli DH5 Alpha. (Plasmid) - pBIG2i	
Approved use of Animals	2007-078-12 for MICE	
Approved Toxin(s)	superantigen, SEE (Staphylococcal Enterotoxin E) - 1 mg maximum Feb 1, 2010 e mail of	

* *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.*

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: J Madrenas

Classification: 2

Date of Last Biohazardous Agents Registry Form: Sep 23, 2009

Date of Last Modification (if applicable):

BioSafety Officer(s): Q Stanley Feb 4, 2010 Ronald Rosenfeld

Chair, Biohazards Subcommittee: G.M. Kidder

USE OF THIS NEW REAGENT:
The SLP-2 protein was identified in a proteomics screening of lipid rafts of activated Jurkat T cells.

The cDNA was generated from these cells and subcloned in pBIG2i with a 3' gfp fusion.

See nucleotide and amino acid sequences attached.

We are currently using this construct to learn about the function of SLP-2.
Specific applications of this reagent are the transfection of the cDNA into the standard cell lines available in the laboratory, the induction of expression ofa chimeric SLP-2-gfp protein, and the biochemical characterization of molecular partners for this protein using standard protein biochemistry techniques.
These experiments are within the standard
operating procedures of a level 2 lab.

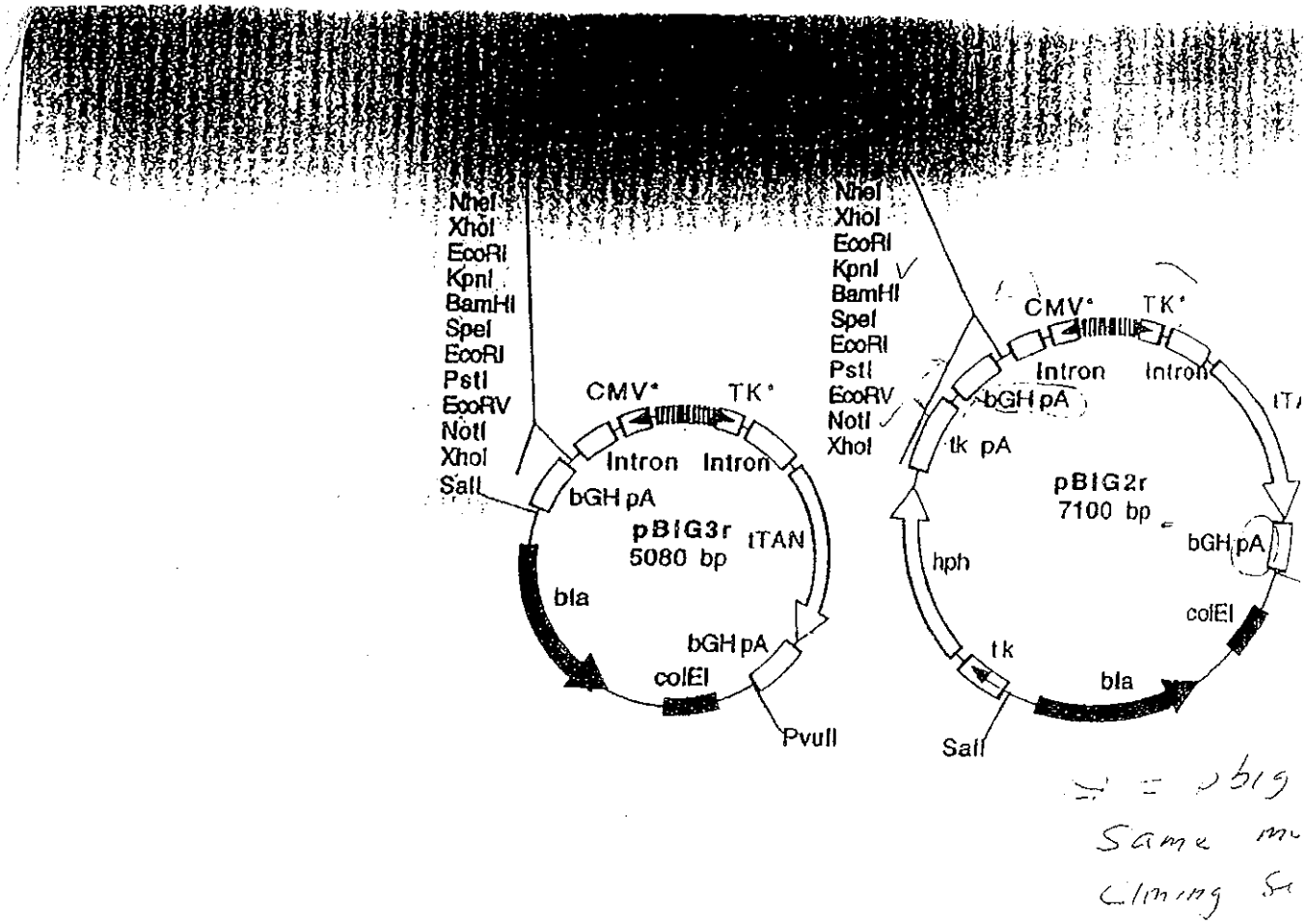


Fig.2: Autoregulated bi-directional tetracycline-responsive pBIG expression vectors. Each vector is based on a high copy number plasmid backbone containing the colE1 origin of replication and B'-lactamase gene that confers resistance to ampicillin. The bi-directional tetracycline-responsive promoter in each vector is comprised of a central tetO element, a stronger CMV* element to drive cDNA expression and a weaker TK* element to drive expression of the transactivator component.

The two vectors are essentially identical with the exception that pBIG2 contains a selectable marker conferring resistance to hygromycin B for the generation of stable cell lines. The "i" series of vectors ulilize the rtTAN transactivator such that cDNA expression is effectively induced by doxycycline.

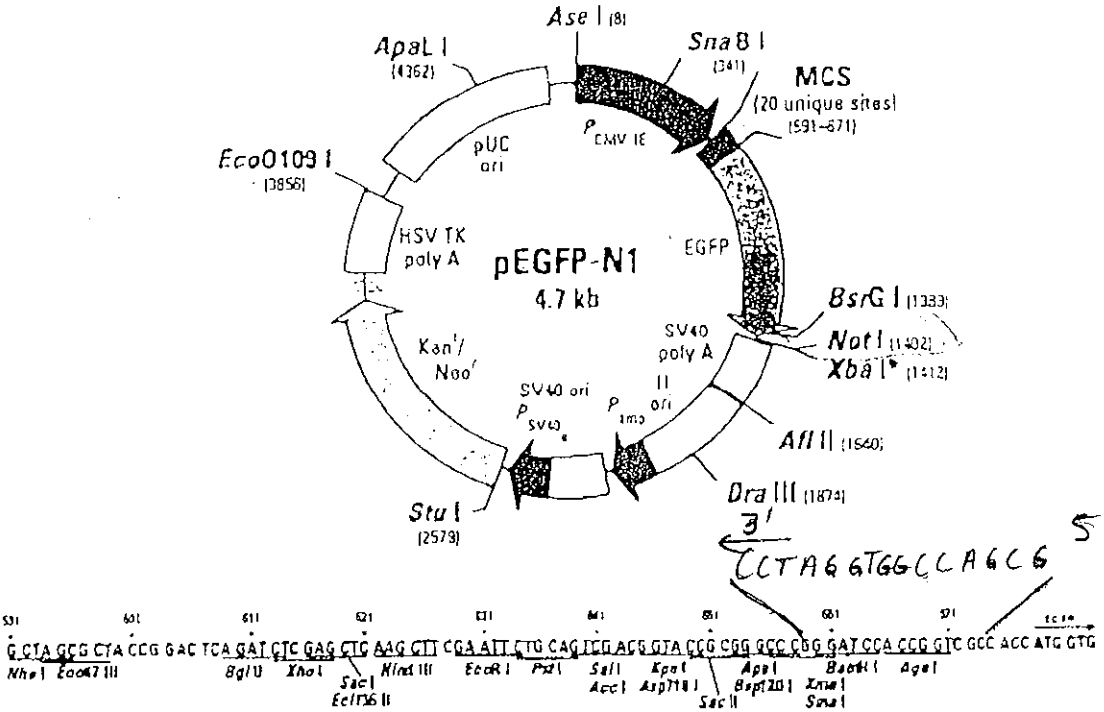
VECTOR INFORMATION

PT3027-5

pEGFP-N1 N-Terminal Protein Fusion Vector

Catalog #6085-1

GenBank Accession #: U55762



Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1. (Unique restriction sites are in bold). The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*$^-$ host and make fresh DNA.

Description:

pEGFP-N1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maxima = 488 nm; emission maxima = 507 nm.) pEGFP-N1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV ($P_{CMV IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex thymidine kinase gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette ($P_{f1 ori}$) expresses kanamycin resistance in *E. coli*. The pGFP-N1 backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR85356)

NCBI Reference Sequence: NM_013442.1

Homo sapiens stomatin (EPB72)-like 2 (STOML2), mRNA

Comment Features Sequence

```
LOCUS       NM_013442               1303 bp    mRNA    linear   PRI
01-NOV-2009
DEFINITION  Homo sapiens stomatin (EPB72)-like 2 (STOML2), mRNA.
ACCESSION   NM_013442
VERSION     NM_013442.1  GI:7305502
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates;
Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1303)
  AUTHORS   Grass,S., Preuss,K.D., Ahlgrimm,M., Fadle,N., Regitz,E.,
            Pfoehler,C., Murawski,N. and Pfreundschuh,M.
  TITLE     Association of a dominantly inherited hyperphosphorylated
            paraprotein target with sporadic and familial multiple
myeloma and
            monoclonal gammopathy of undetermined significance: a
case-control
            study
  JOURNAL   Lancet Oncol. 10 (10), 950-956 (2009)
   PUBMED   19767238
  REMARK    GeneRIF: Familial MGUS and multiple myeloma were
associated with a
            dominant inheritance of hyperphosphorylated paratarg-7
REFERENCE   2  (bases 1 to 1303)
  AUTHORS   Kirchhof,M.G., Chau,L.A., Lemke,C.D., Vardhana,S.,
Darlington,P.J.,
            Marquez,M.E., Taylor,R., Rizkalla,K., Blanca,I.,
Dustin,M.L. and
            Madrenas,J.
  TITLE     Modulation of T cell activation by stomatin-like protein
2
  JOURNAL   J. Immunol. 181 (3), 1927-1936 (2008)
   PUBMED   18641330
  REMARK    GeneRIF: SLP-2 is an important player in T cell
activation by
            ensuring sustained TCR signaling
REFERENCE   3  (bases 1 to 1303)
  AUTHORS   Green,J.B. and Young,J.P.
  TITLE     Slipins: ancient origin, duplication and diversification
of the
            stomatin protein family
  JOURNAL   BMC Evol. Biol. 8, 44 (2008)
   PUBMED   18267007
  REMARK    GeneRIF: Endosymbiotic origin of paraslipin from an
            alphaprotoebacterial ancestor (SLP-2)
            Publication Status: Online-Only
REFERENCE   4  (bases 1 to 1303)
  AUTHORS   Cao,W., Zhang,B., Liu,Y., Li,H., Zhang,S., Fu,L., Niu,Y.,
```

Ning,L.,
Cao,X., Liu,Z. and Sun,B.
TITLE High-level SLP-2 expression and HER-2/neu protein expression are
associated with decreased breast cancer patient survival
JOURNAL Am. J. Clin. Pathol. 128 (3), 430-436 (2007)
PUBMED 17709317
REMARK GeneRIF: High-level SLP-2 expression was associated with decreased
overall survival (P = .011) and was more often found in patients
with tumors larger than 20 mm, lymph node metastasis, advanced
clinical stage, distant metastasis
REFERENCE 5 (bases 1 to 1303)
AUTHORS Cui,Z., Zhang,L., Hua,Z., Cao,W., Feng,W. and Liu,Z.
TITLE Stomatin-like protein 2 is overexpressed and related to cell growth
in human endometrial adenocarcinoma
JOURNAL Oncol. Rep. 17 (4), 829-833 (2007)
PUBMED 17342323
REMARK GeneRIF: SLP-2 was overexpressed in endometrial adenocarcinoma
compared with their normal counterparts.
REFERENCE 6 (bases 1 to 1303)
AUTHORS Guo,D., Han,J., Adam,B.L., Colburn,N.H., Wang,M.H., Dong,Z.,
Eizirik,D.L., She,J.X. and Wang,C.Y.
TITLE Proteomic analysis of SUMO4 substrates in HEK293 cells under serum
starvation-induced stress
JOURNAL Biochem. Biophys. Res. Commun. 337 (4), 1308-1318 (2005)
PUBMED 16236267
REFERENCE 7 (bases 1 to 1303)
AUTHORS Rush,J., Moritz,A., Lee,K.A., Guo,A., Goss,V.L., Spek,E.J.,
Zhang,H., Zha,X.M., Polakiewicz,R.D. and Comb,M.J.
TITLE Immunoaffinity profiling of tyrosine phosphorylation in cancer cells
JOURNAL Nat. Biotechnol. 23 (1), 94-101 (2005)
PUBMED 15592455
REFERENCE 8 (bases 1 to 1303)
AUTHORS Humphray,S.J., Oliver,K., Hunt,A.R., Plumb,R.W., Loveland,J.E.,
Howe,K.L., Andrews,T.D., Searle,S., Hunt,S.E., Scott,C.E.,
Jones,M.C., Ainscough,R., Almeida,J.P., Ambrose,K.D., Ashwell,R.I.,
Babbage,A.K., Babbage,S., Bagguley,C.L., Bailey,J., Banerjee,R.,
Barker,D.J., Barlow,K.F., Bates,K., Beasley,H., Beasley,O.,
Bird,C.P., Bray-Allen,S., Brown,A.J., Brown,J.Y., Burford,D.,
Burrill,W., Burton,J., Carder,C., Carter,N.P., Chapman,J.C.,
Chen,Y., Clarke,G., Clark,S.Y., Clee,C.M., Clegg,S., Collier,R.E.,
Corby,N., Crosier,M., Cummings,A.T., Davies,J., Dhami,P., Dunn,M.,
Dutta,I., Dyer,L.W., Earthrowl,M.E., Faulkner,L., Fleming,C.J.,
Frankish,A., Frankland,J.A., French,L., Fricker,D.G., Garner,P.,
Garnett,J., Ghori,J., Gilbert,J.G., Glison,C., Grafham,D.V.,
Gribble,S., Griffiths,C., Griffiths-Jones,S., Grocock,R., Guy,J.,
Hall,R.E., Hammond,S., Harley,J.L., Harrison,E.S., Hart,E.A.,
Heath,P.D., Henderson,C.D., Hopkins,B.L., Howard,P.J., Howden,P.J.,
Huckle,E., Johnson,C., Johnson,D., Joy,A.A., Kay,M., Keenan,S.,
Kershaw,J.K., Kimberley,A.M., King,A., Knights,A., Laird,G.K.,
Langford,C., Lawlor,S., Leongamornlert,D.A., Leversha,M., Lloyd,C.,
Lloyd,D.M., Lovell,J., Martin,S., Mashreghi-Mohammadi,M.,
Matthews,L., McLaren,S., McLay,K.E., McMurray,A., Milne,S.,
Nickerson,T., Nisbett,J., Nordsiek,G., Pearce,A.V., Peck,A.I.,
Porter,K.M., Pandian,R., Pelan,S., Phillimore,B., Povey,S.,
Ramsey,Y., Rand,V., Scharfe,M., Sehra,H.K., Shownkeen,R.,
Sims,S.K., Skuce,C.D., Smith,M., Steward,C.A., Swarbreck,D.,
Sycamore,N., Tester,J., Thorpe,A., Tracey,A., Tromans,A.,
Thomas,D.W., Wall,M., Wallis,J.M., West,A.P., Whitehead,S.L.,
Willey,D.L., Williams,S.A., Wilming,L., Wray,P.W., Young,L.,

Related sequences
Full text in PMC
GEO profiles
Gene
Gene genotype
GeneView in dbSNP
Genome
HomoloGene
Map viewer
Master
OMIM
Order cDNA clone
Probe
Protein
PubMed
PubMed (RefSeq)
PubMed (weighted)
SNP
Taxonomy
UniGene
UniSTS
mRNA genome project
LinkOut

Ashurst,J.L., Coulson,A., Blocker,H., Durbin,R., Sulston,J.E., Hubbard,T., Jackson,M.J., Bentley,D.R., Beck,S., Rogers,J. and Dunham,I.
TITLE DNA sequence and analysis of human chromosome 9
JOURNAL Nature 429 (6990), 369-374 (2004)
PUBMED 15164053
REFERENCE 9 (bases 1 to 1303)
AUTHORS Owczarek,C.M., Treutlein,H.R., Portbury,K.J., Gulluyan,L.M., Kola,I. and Hertzog,P.J.
TITLE A novel member of the STOMATIN/EPB72/mec-2 family, stomatin-like 2 (STOML2), is ubiquitously expressed and localizes to HSA chromosome 9p13.1
JOURNAL Cytogenet. Cell Genet. 92 (3-4), 196-203 (2001)
PUBMED 11435687
REFERENCE 10 (bases 1 to 1303)
AUTHORS Wang,Y. and Morrow,J.S.
TITLE Identification and characterization of human SLP-2, a novel homologue of stomatin (band 7.2b) present in erythrocytes and other tissues
JOURNAL J. Biol. Chem. 275 (11), 8062-8071 (2000)
PUBMED 10713127
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final NCBI review. The reference sequence was derived from AF190167.1.

Publication Note: This RefSeq record includes a subset of the publications that are available for this gene. Please see the Entrez Gene record to access additional publications.

FEATURES Location/Qualifiers

source 1..1303
/organism="Homo sapiens"
/mol_type="mRNA"
/db_xref="taxon:9606"
/chromosome="9"
/map="9p13.1"

gene 1..1303
/gene="STOML2"
/gene_synonym="HSPC108; SLP-2"
/note="stomatin (EPB72)-like 2"
/db_xref="GeneID:30968"
/db_xref="HGNC:14559"
/db_xref="HPRD:16310"
/db_xref="MIM:608292"

exon 1..108
/gene="STOML2"
/gene_synonym="HSPC108; SLP-2"
/inference="alignment:Splign"
/number=1

CDS 64..1134
/gene="STOML2"
/gene_synonym="HSPC108; SLP-2"
/note="stomatin-like protein 2; stomatin-like 2"
/codon_start=1
/product="stomatin (EPB72)-like 2"
/protein_id="NP_038470.1"
/db_xref="GI:7305503"
/db_xref="CCDS:CCDS6577.1"
/db_xref="GeneID:30968"
/db_xref="HGNC:14559"
/db_xref="HPRD:16310"
/db_xref="MIM:608292"
/translation="MLARAARGTGALLLRGSLLASGRAPRRASSGLPRNTVVLFVPQQ
EAWVVERMGRFHRILEPGLNILIPVLDRIRYVQSLKEIVINVPEQSAVTLDNVTLQID
GVLYLRIMDPYKASYGVEDPEYAVTQLAQTTMRSELGKLSLDKVFRERESLNASIVDA
INQAADCWGIRCLRYEIKDIHVPPRVKESMQMQVEAERRKRATVLESEGTRESAINVA
EGKKQAQILASEAEKAEQINQAAGEASAVLAKAKAKAEAIRILAAALTQHNGDAAASL
TVAEQYVSAFSKLAKDSNTILLPSNPGDVTSMVAQAMGVYGALTKAPVPGTPDSLSSG
SSRDVQGTDASLDEELDRVKMS"

exon 109..246
/gene="STOML2"

```
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=2
     exon            247..346
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=3
     exon            347..405
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=4
     exon            406..507
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=5
     exon            508..642
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=6
     exon            643..787
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=7
     exon            788..867
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=8
     exon            868..996
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=9
     exon            997..1278
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /inference="alignment:Splign"
                     /number=10
     STS             1041..1192
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /standard_name="A002P16"
                     /db_xref="UniSTS:43594"
     STS             1069..1200
                     /gene="STOML2"
                     /gene_synonym="HSPC108; SLP-2"
                     /standard_name="RH41785"
                     /db_xref="UniSTS:12941"
ORIGIN
        1 ggcttctggg agcgaccgct ccgctcgtct cgttggttcc ggaggtcgct gcggcggtgg
       61 gaaatgctgg cgcgcgcggc gcggggcact ggggcccttt tgctgagggg ctctctactg
      121 gcttctggcc gcgctccgcg ccgcgcctcc tctggattgc cccgaaacac cgtggtactg
      181 ttcgtgccgc agcaggaggc ctgggtggtg gagcgaatgg gccgattcca ccggatcctg
      241 gagcctggtt tgaacatcct catccctgtg ttagaccgga tccgatatgt gcagagtctc
      301 aaggaaattg tcatcaacgt gcctgagcag tcggctgtga ctctcgacaa tgtaactctg
      361 caaatcgatg gagtccttta cctgcgcatc atggaccctt acaaggcaag ctacggtgtg
      421 gaggaccctg agtatgccgt cacccagcta gctcaaacaa ccatgagatc agagctcggc
      481 aaactctctc tggacaaagt cttccgggaa cgggagtccc tgaatgccag cattgtggat
      541 gccatcaacc aagctgctga ctgctggggt atccgctgcc tccgttatga gatcaaggat
      601 atccatgtgc caccccgggt gaaagagtct atgcagatgc aggtggaggc agagcggcgg
      661 aaacgggcca cagttctaga gtctgagggg acccgagagt cggccatcaa tgtggcagaa
      721 gggaagaaac aggcccagat cctggcctcc gaagcagaaa aggctgaaca gataaatcag
      781 gcagcaggag aggccagtgc agttctggcg aaggccaagg ctaaagctga agctattcga
      841 atcctggctg cagctctgac acaacataat ggagatgcag cagcttcact gactgtggcc
```

```
      901 gagcagtatg tcagcgcgtt ctccaaactg gccaaggact ccaacactat cctactgccc
      961 tccaaccctg gcgatgtcac cagcatggtg gctcaggcca tgggtgtata tggagccctc
     1021 accaaagccc cagtgccagg gactccagac tcactctcca gtgggagcag cagagatgtc
     1081 cagggtacag atgcaagtct tgatgaggaa cttgatcgag tcaagatgag ttagtggagc
     1141 tgggcttggc cagggagtct ggggacaagg aagcagattt tcctgattct ggctctagct
     1201 tccctgccaa gattttggtt tttatttttt tatttgaact ttagtcgtgt aataaactca
     1261 ccagtggcaa acctgaaaaa aaaaaaaaaa aaaaaaaaaa aaa
//
```

Subject: Re: Toxin Order
From: "Dr. J. (Quim) Madrenas" <madrenas@robarts.ca>
Date: Thu, 04 Feb 2010 10:58:49 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: rsn@uwo.ca, Luan Chau <luan@robarts.ca>

Thank you very much Jennifer. Greatly appreciated.

2

On 04/02/10 10:24 AM, "Jennifer Stanley" <jstanle2@uwo.ca> wrote:

> Hi Dr. Madrenas:
> Your purchase order has been approved and that Ron is working on the
> PHAC and CFIA paperwork for Cedarlane.
> Please note that this amount (1 mg) should be the maximum that you keep
> on hand in the laboratory due to biosecurity requirements.
> Regards,
> Jennifer

--
J. (Quim) Madrenas, MD PhD
Canada Research Chair in Immunobiology
Professor, Microbiology & Immunology, and Medicine
The University of Western Ontario
Head of Immunology, Robarts Research Institute
Director, FOCIS Centre for Clinical Immunology and Immunotherapeutics

Robarts Research Institute
Room 2.C5, P.O. Box 5015, 100 Perth Drive
London, Ontario
Canada N6A 5K8
Telephone: (519) 663-3777, ext.: 24242
FAX: (519) 931-5268
http://www.robarts.ca/madrenas

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents 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 biohazards 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 Biohazard 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: DR. J. MADRENAS
SIGNATURE: Jmadrenas
DEPARTMENT: ROBARTS RESEARCH INST / IMMUNOLOGY
ADDRESS: 100 PERTH DRIVE, LONDON, ON
PHONE NUMBER: (519) 663-5777 ext 24211
EMERGENCY PHONE NUMBER(S): (519) 679-6862
EMAIL: madrenas@robarts.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 2278 & 2276

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): 1- The role of SLP-2 in TCR Signalling
2- Regulation of CTLA4 Function

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

LUAN A. CHAU
THU A. CHAU
DARAH CHRISTIE
SAMAR SAYEDYAHOSSEIN
ISAAC ELIAS
SARA RAMOS

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☑YES ○ NO
(including but not limited to 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)*	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
SUPERANTIGEN	☑Yes ○ No	☑Yes ○ No	○ Yes ☑No	1 microliter	STAPH. AUREUS	○ 1 ☑2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 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 ○ No	Human blood + human cell lines	Not applicable
Rodent	☑Yes ○ No	Mice	
Non-human primate	○ Yes ☑No		
Other (specify)	○ Yes ☑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)*	Supplier / Source
Human	☑ Yes ○ No	E6.1 Jurkats HEK 293	ATCC
Rodent	○ Yes ○ No		
Non-human primate	○ Yes ○ No		
Other (specify)	○ Yes ○ No		

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

2.4 For above named cell types(s) indicate PHAC 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 ○ NO
If no, please proceed to Section 4.0

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	NORMAL Volunteer Donors	○ Yes ☑ No		○ 1 ☑ 2 ○ 3
Human Blood (fraction) or other Body Fluid	Peritoneal Fluids from PD Patients	☑ Yes ○ No	S. Aureus	○ 1 ☑ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. Coli DH-5α	pBIG2i	Internal Source	CTLA4 SLP 2	none

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ☑ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used _____
Please attach a full description of the biological agent.

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used MICE

6.3 AUS protocol # 2007-078-12

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

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin unknown for humans (please see attached note!)

8.4 How much of the toxin is handled at one time*? < 0.5ug

8.5 How much of the toxin is stored*? ~~50mg~~ 1 mg maximum JF - Feb 1, 2010 (per email).

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

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

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____

_____

_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____

_____

_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES O NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree..)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no please proceed to Section 12.0 O NO

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

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

SIGNATURE _____

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus BIO-RBI-0020
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: August 31/2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 23 Sept. 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: September 01, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: Sept 23/09

Approval Number: BIO-RBI-0020 Expiry Date (3 years from Approval): September 22, 12

Special Conditions of Approval:

1) Follow the "Biosecurity Requirements for Facilities Using Biological Agents" attached. See uwo.ca/humanresources/biosafety for information

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

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

8.3

The LD50 for staphylococcal superantigens for humans is not known. Preliminary studies using non-human primates suggested that it may be in the range of 0.02micrograms/Kg (1.4 micrograms for a normal 70Kg adult) when using inhalatory route. To further minimize the risk, we will aliquot our stocks to a maximum of 1 microgram per aliquot . Note that we never use more than 0.5 microgram per experiment. Also, all out stocks are stored under lock and key and under strict control of my chief technician Ms. Luan Chau.

Ron Noseworthy

From: madrenas [madrenas@robarts.ca]
Sent: September 22, 2009 6:30 AM
To: john McCormick; luan@robarts.ca; Ron Noseworthy
Cc: madrenas
Subject: SAgs

Thanks John, Luan and Ron,

The reference I got for the LD50 in monkeys by inhalatory route is the one from the US Army Manual. In the context of the tenuous and heterogenous data you reviewed in your mail, I believe that the LD50 in this paper is the closest we have as a proper reference. We should though qualify the claim indicating that the evidence for human LD50 is not available, that the evidence for non-human primates is very limited, and that there are very substantial species differences in terms of sensitivity to SAgs. Altogether, the LD50 may be not applicable as such to human beings.

Q

Fwd: Re: Biosafety form

Ron Noseworthy

From: Luan A. Chau [luan@robarts.ca]
Sent: September 21, 2009 11:06 AM
To: Ron Noseworthy
Cc: Quim Madrenas
Subject: Fwd: Re: Biosafety form

Dear Ron,

Please see below.

Thanks,
Luan

From: John McCormick <john.mccormick@schulich.uwo.ca>
To: "Luan A. Chau" <luan@robarts.ca>, Quim Madrenas <madrenas@robarts.ca>
Subject: Re: Biosafety form
Date: Mon, 21 Sep 2009 10:42:09 -0400

Hi Luan and Quim,

I haven't been able to find the data described below for the 1.4 microgram/70 kg adult by inhalation. But after some digging, this is what I did find:

The LD50 for bacterial (staphylococcal enterotoxins) superantigens is not known. However, 3 historical events in the early 1960s from the now disbanded U.S. Offensive Biological Warfare Program indicates that accidental inhalation of staphylococcal enterotoxin B in the microgram range may result in symptoms of fever, cough, nausea and vomiting (among other symptoms), although there were no deaths (Rusnak et al., 2004 Emerg Infect Dis). There are no further documented reports of any additional aerosol exposures despite 5000 research publications on superantigens since 1970. In addition, aerosol models indicated that a "lethal dose" in monkeys was 190 micrograms/kg body weight (Tseng et al., 1993 Infect Immun). An LD50 by this route is probably closer to ~10-20 micrograms/kg although this was not made clear (Tseng et al. 1995 Infect Immun).

Feb 1/10
1 mg max per e-mail QI

LD 10 ng/kg (conservative) x 50 kg (small person) = 500 ng, ~450 mg on hand

In addition, anti-cancer clinical Phase I trials using wild-type staphylococcal enterotoxin A (fused with a monoclonal antibody to target cancer cells) infused over 3 hours as a single dose is safe when given at doses up to 4 nanograms/kg (total dose ~ 280 ng for a 70 kg adult) (Nielsen et al., 2000 J. Immunother). Infusion of a mutated version of the therapeutic indicated that the maximum tolerated dose (MTD) ranged from 103 ng/kg to 601 ng/kg infused per day for 4 days (~7 micrograms to 42 micrograms per day x 4 days) (Cheng et al., 2004 J. Clin Oncol).

I think the evidence is clear we should be careful not to inhale these things, and that a dose to induce symptoms would probably be in the low microgram range, but I also think an actual lethal dose would be quite high.

Fwd: Re: Biosafety form

Hope this helps.

John.

On 18-Sep-09, at 3:57 PM, Luan A. Chau wrote:

Thanks John. Have a great weekend!

Luan

Hi Luan,

I am working on getting you the references for human dose. I'm not sure I believe the inhalation doses are accurate however so please don't submit this information yet. Thanks,

John.

On 18-Sep-09, at 10:44 AM, Luan A. Chau wrote:

Dear John,

For our Biohazard Registry Form renewal, the reference for the LD50 as we claimed below needs to be included with the form.

Could you please send me the reference for this.

Thanks very much for your help.

Best regards,

Luan

Dear Luan,

For the biosafety form, John McC told me that he did answer just that it was not known for humans and that mouse strains are mostly resistant to superantigens. For the few that are susceptible, it was on the range of 100 to 500 micrograms.

My understanding is that we have to put it for humans. If so, please include the information below in the requested point (I believe it is 8.3). Show it to Ron Noseworthy before submission for approval.

The LD50 for staphylococcal superantigens for humans is not known. Preliminary studies using non-human primates suggested that it may be in the range of 0.02micrograms/Kg (1.4 micrograms for a normal 70Kg adult) when using inhalatory route. To further minimize the risk, we will aliquot our stocks to a maximum of 1 microgram per aliquot . Note that we never use more than 0.5 microgram per experiment. Also, all out stocks are stored under lock and key and under strict control of my chief technician Ms. Luan Chau.

Q

--

J. (Quim) Madrenas, MD PhD
Canada Research Chair in Immunobiology
Professor, Microbiology & Immunology, and Medicine
The University of Western Ontario
Head of Immunology, Robarts Research Institute
Director, FOCIS Centre for Clinical Immunology and Immunotherapeutics

Robarts Research Institute
Room 2.05, P.O. Box 5015, 100 Perth Drive
London, Ontario
Canada N6A 5K8
Telephone: (519) 663-5777, ext.: 24242
FAX: (519) 931-5268
http://www.robarts.ca/madrenas

Luan Ai Chau, HBSc.
Research Assistant
Immunology / Madrenas Lab
University of Western Ontario
Robarts Research Institute
Room 2278, P.O. Box 5015,
100 Perth Drive,
London, Ontario, Canada N6A 5K8
Phone: (519) 663-5777, ext. 24211
FAX: (519) 931-5268

--

Luan Ai Chau, HBSc.
Research Assistant
Immunology / Madrenas Lab
University of Western Ontario
Robarts Research Institute
Room 2278, P.O. Box 5015,
100 Perth Drive,
London, Ontario, Canada N6A 5K8
Phone: (519) 663-5777, ext. 24211
FAX: (519) 931-5268

--

Luan Ai Chau, HBSc.
Research Assistant
Immunology / Madrenas Lab
University of Western Ontario
Robarts Research Institute
Room 2278, P.O. Box 5015,
100 Perth Drive,
London, Ontario, Canada N6A 5K8
Phone: (519) 663-5777, ext. 24211
FAX: (519) 931-5268

SYSTEMS IMMUNOLOGY OF *S. aureus* INFECTION

Overview: The goal of this research program is to develop a Systems Immunology of the human response to *Staphylococcus aureus* (*S. aureus*).

Background and preliminary data: During the previous grant term, we examined the regulation of human T cell activation by CTLA-4 using the response to *S. aureus* superantigens as a model. In the course of that work, we unraveled novel aspects of human T cell activation by these toxins such as the regulatory role of Lck (*J Immunol* 2004; 172: 222) and a new Gα11-PLCβ-dependent activation pathway (*Immunity* 2006; 25:67). More important, we identified a new mechanism used by *S. aureus* to down-regulate T cell activation by superantigens (*Nature Medicine* 2009; 15: 641). This mechanism is operational *in vivo* and may explain the balance between commensalism and pathogenicity by *S. aureus*. Preliminary data indicate that this mechanism involves binding of staphylococcal peptidoglycan (PGN)-embedded molecules to TLR2 complexes on antigen-presenting cells (APCs), and the induction of an NF-κB-dependent, interleukin-10 response leading to down-regulation of T cell response to superantigens. In addition, recent experiments suggest that the modulatory effects of staphylococcal PGN-embedded molecules is dependent on its selective binding to TLR2/6, but not TLR2/1, complexes on APCs. The specific focus of this grant is the comprehensive dissection of the molecular basis of such a mechanism.

Hypothesis: Selective binding of staphylococcal PGN-embedded molecules to TLR2/6 on APCs triggers unique genomic and proteomic profiles in these cells leading to modulation of the immune response to *S. aureus*.

Specific aims:

1. To identify the factors that control selective binding of PGN-embedded molecules to TLR2/6;
2. To build the signaling network involved in immunomodulation by staphylococcal PGN-embedded molecules;
3. To establish the genomic and proteomic profiles in APCs and in T cells during immunomodulation by *S. aureus* PGN preparations;
4. To generate a computational model of immunomodulation by the staphylococcal cell wall; and
5. To test the data network in a clinical setting of sepsis and shock by *S. aureus*.

Experimental approach: We will follow a systems approach to study the complex temporal and spatial interactions between APCs and T cells during the response to *S. aureus* superantigens in the presence or absence of staphylococcal PGN preparations. First, we will assess basal and ligand-induced TLR2/6 vs. TLR2/1 dimerization with varying amounts of each chain and in different monocyte subsets vs. monocyte-derived macrophages vs. monocyte-derived dendritic cells and correlate this with functional modulation of T cell responses to superantigens. Next, we will examine the activation of signaling pathways emanating from TLR2/6 and link their activation with modulation of T cell activation. Once the optimal cellular and biochemical conditions of immunomodulation have been identified, we will perform genomic and proteomic

analyses of the responding cells. The resulting body of data will undergo bioinformatic analysis and will be fed into a computer mode that we have already started to build to predict the course of immune responses to staphylococcal superantigens. Predictions from this model will be tested in the clinic using peripheral blood cells from patients in ICU with *S. aureus* sepsis vs. shock, and iterative model refinements will be performed.

Relevance: *S. aureus* poses a paradox: on one hand, it is carried by up to 50% of healthy individuals but on the other hand, it is also one of the most common pathogens in the clinic. How *S. aureus* can act as a commensal or as a pathogen is not known. The proposed work will identify molecular profiles associated with commensalism and pathogenicity, and reveal potential therapeutic targets to act as alternatives to antibiotics.

Toxin Technology, Inc.

7165 Curtiss Ave
Sarasota, FL 34231
USA

941-925-2032 (ph)
941-925-2136 (fax)
Email: toxtech@att.net

Certificate of Analysis

Product : Staphylococcal Enterotoxin E, partially purified

cat. no : EP404

lot no. : 31301Pe

purity : approximately 50 % pure by SDS-PAGE Coomassie Blue stain

serological : 10 ug / ml solution showed lines of identity with 10 ug SEE / ml standard when tested with anti SEE in double immunodiffusion assay. No cross reactivity was observed when tested at a 100ug/ml with anti SEA, SEB, SEC, SED and TSST (sensitivity approx. 5 ug / ml)

solubility : After lyophilization, the SEE was re-dissolved to a 1 mg / ml solution using deionized water. This solution was clear within several minutes.

storage : At - 20 °C, 1 mg / ml solution is stable for one year under serological freezer conditions.
At 4 °C, 1 mg / ml solution is stable for two weeks.
In lyophilized, desiccated form - stable for at least 5 years

This product is for research purposes only and is not intended for in vivo or diagnostic use

Toxin Technology shall not be held responsible for any damages resulting from the use of this product

TOXIN TECHNOLOGY, INC.

7155 CURTISS AVENUE • SARASOTA, FLORIDA 34231
PHONE (941) 925-2032 • FAX (941) 925-2130 • Email: toxtech@worldnet.att.net

R.F. REISER, Ph.D. "Tox Tech" R.H. REISER, Ph.D.

HEALTH HAZARD DATA

To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

OPEN AND REHYDRATE VIALS IN BIOSAFETY SHEET.

Acute effects
May be harmful if swallowed, inhaled, or absorbed through skin.
Biomedical material. May cause human disease.
Causes emesis and diarrhea in experimental animals.
Associated with food poisoning and causes enteritis in humans. The dose of purified protein required to produce emesis or diarrhea in monkeys is 0.9ug/kg by oral feeding (Biochem. Vol. 4, 1965).

Aerosols may be harmful; 30ng/person (incapacitating), 1.7ug/person may be lethal)! Re-hydrate lyophilized toxins in bio-safety hood. Once rehydrated, handle liquid in chemical hood or bio-safety hood when mixing or agitating.

FIRST AID

In case of contact, flush with copious amounts of water. If swallowed, induce vomiting then wash mouth with water provided person is conscious. Call a physician.

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.

If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

FIRE AND EXPLOSION HAZARD

Use extinguishing media appropriate for surrounding fire.

Firefighters should wear proper protective equipment and self-contained breathing apparatus
with full facepiece.

1. Franz, DR et al Clinical Recognition and Management of Patients Exposed to Biological
Warfare Agents.1997. JAMA 278(5):399-411.

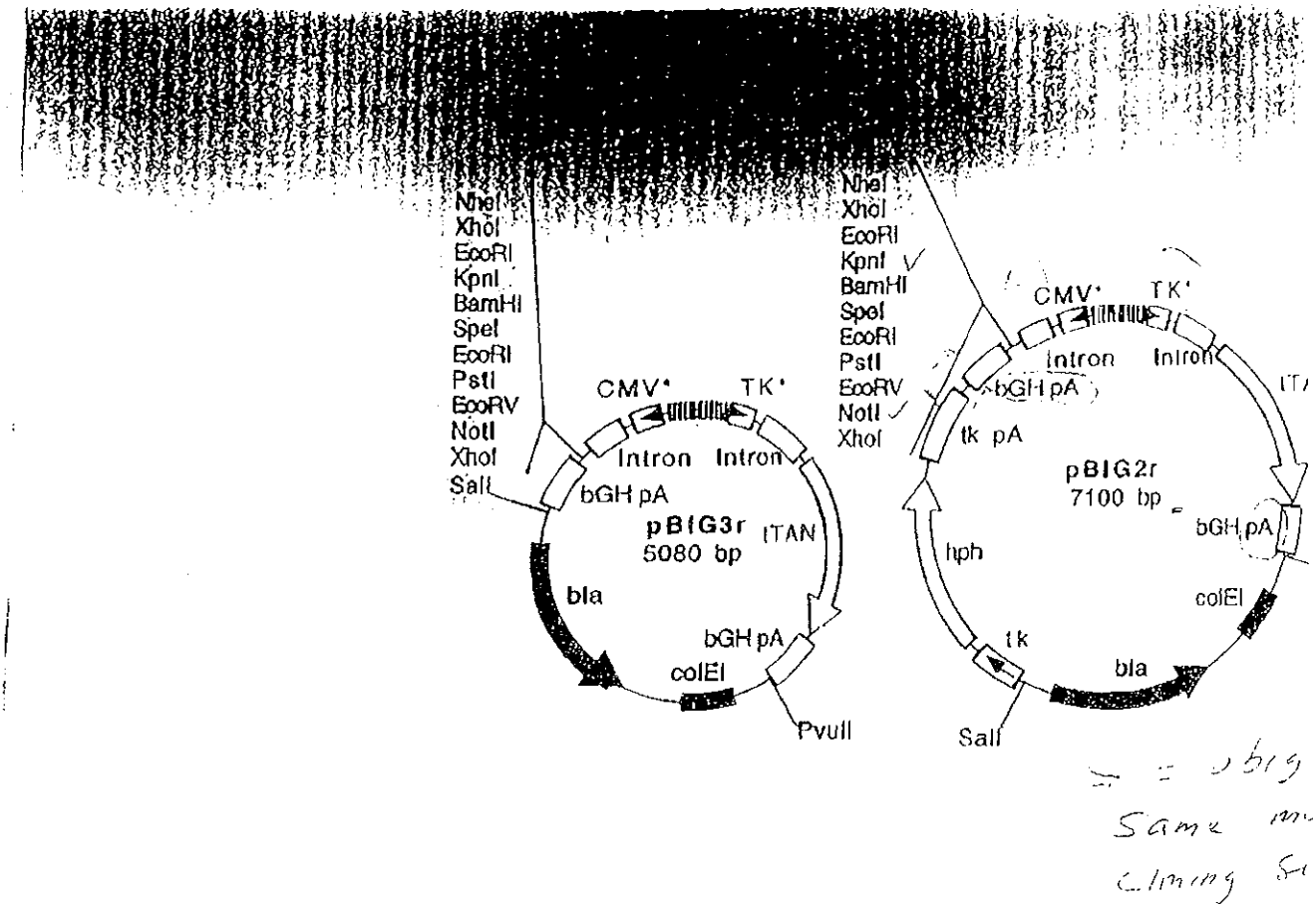


Fig.2: Autoregulated bi-directional tetracycline-responsive pBIG expression vectors. Each vector is based on a high copy number plasmid backbone containing the colE1 origin of replication and B'-lactamase gene that confers resistance to ampicillin. The bi-directional tetracycline-responsive promoter in each vector is comprised of a central tetO element, a stronger CMV* element to drive cDNA expression and a weaker TK* element to drive expression of the transactivator component.

The two vectors are essentially identical with the exception that pBIG2 contains a selectable marker conferring resistance to hygromycin B for the generation of stable cell lines. The "i" series of vectors ulilize the rtTAN transactivator such that cDNA expression is effectively induced by doxycycline.

Cell Biology

ATCC® Number:	**TIB-152™** Order this item	Price:	**$264.00**
Designations:	Jurkat, Clone E6-1		
Depositors:	A Weiss		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	suspension		
Organism:	*Homo sapiens* (human)		
Morphology:	lymphoblast 		
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;		
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]		
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 (technology from amaxa Roche FuGENE® Transfection Reagents)		
Receptors:	T cell antigen receptor, expressed		
Antigen Expression:	CD3; Homo sapiens, expressed		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18		
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.		

Related Links ▸

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

Gender:	male
Comments:	This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609] The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530] Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609] The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml. **Interval:** Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	derivative:ATCC CRL-1990 derivative:ATCC CRL-2063 recommended serum:ATCC 30-2020 derivative:ATCC TIB-153 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001

Cell Biology

ATCC® Number:	CRL-1573™ Order this Item	Price:	$256.00
Designations:	293 [HEK-293]		
Depositors:	FL Graham		
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial 		
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA		
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.		
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.		
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]		
Receptors:	vitronectin, expressed		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19		

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

Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus
Comments:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406] The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Preservation:

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006