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
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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 O 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? O 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 O No	☑Yes O No	O Yes ☑No	1 microliter	STAPH, AUREUS	O 1 ☑2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 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 O 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 O No	-Human blood -Human cell lines	Not applicable
Rodent	☑Yes O No	-Mice	
Non-human primate	O Yes ☑No		
Other (specify)	O Yes ☑No		

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

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	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) 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 _____

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

8.5 How much of the toxin is stored*? 50mg

*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 life stage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

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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.

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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: _____

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

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

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

Special Conditions of Approval:

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

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 model 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)
941-925-2130 (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.

7165 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.

MATERIAL SAFETY DATA SHEET

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(S):399-411.

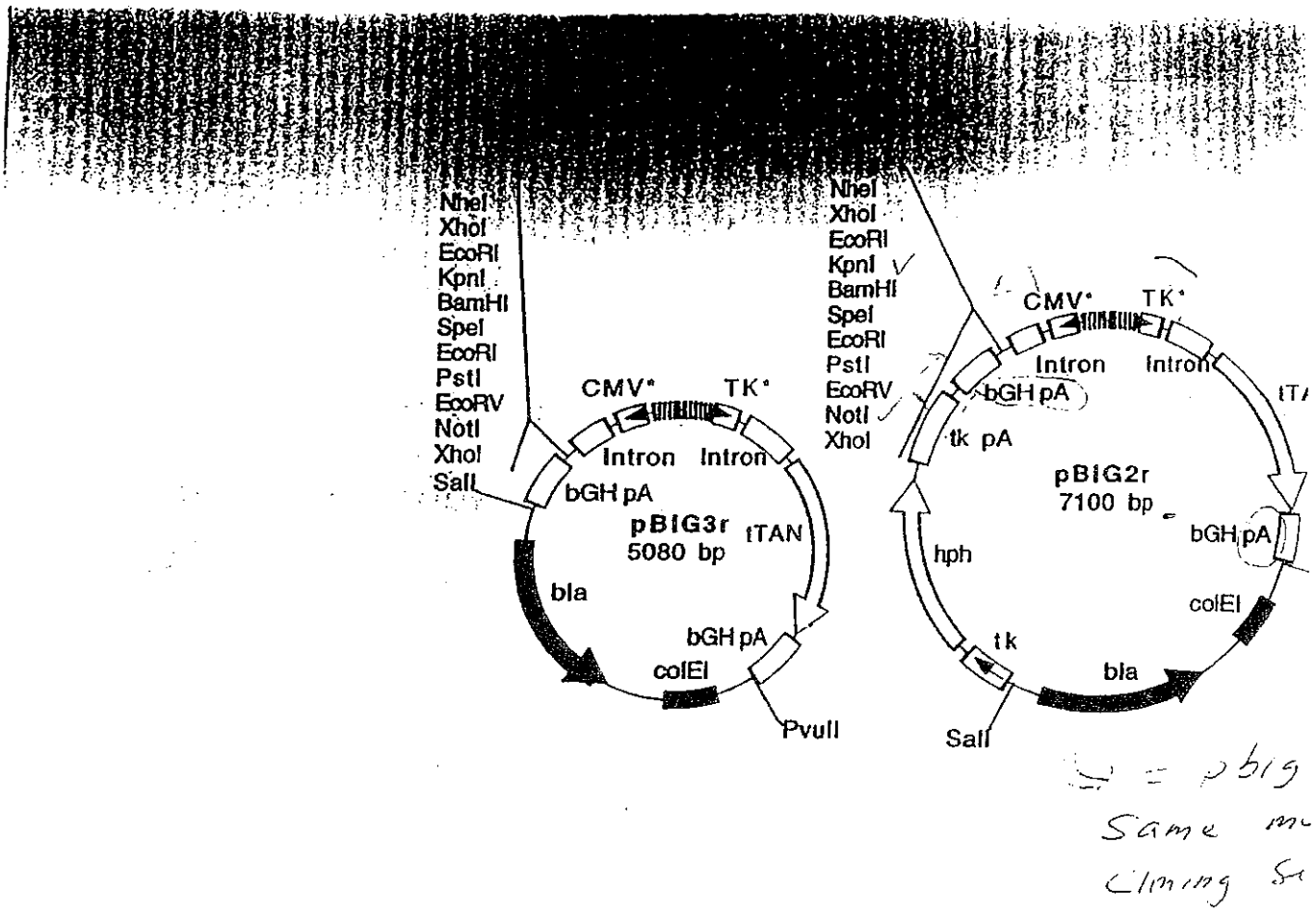

Fig.2: Autoregulated bi-directional tetracycline-responsive pBIG expression vectors. Each vector is based on a high copy number plasmid backbone containing the colE1 origin of replication and B'-lactamase gene that confers resistance to ampicillin. The bi-directional tetracycline-responsive promoter 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] **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%.
Propagation:	**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.

Subculturing:

Protocol:

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