

**THE UNIVERSITY OF WESTERN ONTARIO
 BIOHAZARDOUS AGENTS REGISTRY FORM**
 Approved Biohazards Subcommittee: July 25, 2008
 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 are 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits. The form must also be completed if any work is proposed involves plants or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

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, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Weiping Min
 SIGNATURE [Signature]
 DEPARTMENT Surgery
 ADDRESS UW, C9-136
 PHONE NUMBER 32985
 EMAIL minweiping@uwo.ca

Location of experimental work to be carried out: Building(s) LHSC- U4 Room(s) C9-119

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CBCF
 GRANT TITLE(S): Treatment of Breast Cancer through RAI

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.

See attachment

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

Xiufen Zheng
Xusheng Zhang
Marianne Beddo
Aminah Shunnar
Di Chen

* 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 microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? YES NO

If no, please proceed to Section 2.0

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	Health Canada or CFIA Containment Level
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No	Mouse Bone Marrow	2009-cob.
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

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
Human	<input type="radio"/> Yes <input type="radio"/> No		
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	4T1 B16. P815	ATCC
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

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

2.4 For above named cell types(s) indicate 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 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)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved? YES, please specify _____ NO

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

(Not involved in this project)

5.1 Will human clinical trials be conducted using the viral vector in 4.0? YES 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, number: _____ NO PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used C57BL/6 Balb/c DBA, SCID

6.3 AUS protocol # 2009-006

6.4 Will any of the agents listed be used in live animals YES, specify: 20 lines NO
(+T1, B16, p35 cells)

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 YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES NO
- ◆ Non- Human Primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

8.0 Biological Toxins

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

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

8.3 What is the LD₅₀ (specify species) of the toxin _____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? 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? Initiate and maintain colony, give location: _____
 "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:

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? YES 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? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES 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? YES NO

10.9 Please describe any CFIA permit conditions:

11.0 Import Requirements

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

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

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

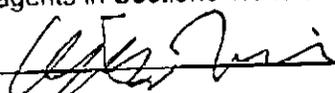
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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.

SIGNATURE 

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

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. 1 2 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus _____
 NO
 NOT REQUIRED

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 have an up-to-date Position Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE *[Signature]* Date: Jan 22, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: *[Signature]*
Date: Feb 2, 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:

Brief description of work

Tumor-derived immune suppression is a major impediment to successful immune/gene cancer therapy. Here we describe a novel strategy to disrupt tumor-derived immune suppression by silencing a tolerogenic molecule of tumor origin, indoleamine 2,3-dioxygenase (IDO), using small interfering RNA (siRNA). Silencing of IDO in B16F10 cells in vitro using IDO-siRNA prevented catabolism of tryptophan and inhibited apoptosis of T cells. IDO-siRNA treatment of B16F10 cells in vitro inhibited subsequent growth, tumor formation and the size of tumor formed, by those cells when transplanted into host mice. In vivo treatment of B16F10 tumor-bearing mice successfully postponed tumor formation time and significantly decreased tumor size. Furthermore, in vivo IDO-siRNA treatment resulted in recovery of T cells responses and enhancement of tumor-specific killing. Thus, silencing IDO may break tumor-derived immune suppression. These data indicate that RNA interference has potential to enhance cancer therapy by reinstalling anti-cancer immunity. We also found immunize mice with IDO-silenced Dc before tumour challenge can delay tumour onsetting.

Our Hypothesis is inhibiting IDO using siRNA will enhance anti-tumour effect and improve the anti-tumour therapies.

Tumor cells (4T1, B16, PB15) will be cultured in vitro. Mice will be injected with 1×10^5 (in 100ul of PBS) tumour cells in one site (Breast cancer cell in fat pad) or Melanoma cancer cells (s.c.). Mice will be treated with IDO siRNA or IDO silenced DC i.v. before or after tumour cell injection. the tumour size will be measured every other day to observe tumour growth.

ATCC**MATERIAL SAFETY DATA SHEET****MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)**

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)

Chemtrec: (800) 424-9300

To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I**Hazardous Ingredients**

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II**Physical data**

Pink or red aqueous liquid

SECTION III**Health hazards****For Biosafety Level 1 Cell Lines**

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV**Fire and explosion**

Not applicable

ATCC**MATERIAL SAFETY DATA SHEET****SECTION V****Reactivity data**

Stable. Hazardous polymerization will not occur.

SECTION VI**Method of disposal**

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all federal, State and local regulations.

SECTION VII**Special protection information****For Biosafety Level 1 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII**Special precautions or comments**

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the **Journal of Tissue Culture Methods** (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

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February 2002

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108

Emergency Telephone: (703) 365-2710 (24 hours)
Information Telephone: (703) 365-2704
Chemtrec (800) 424-9300

Cell Biology

ATCC® Number:	CRL-2539™	Order this Item	Price:	\$268.00
Designations:	4T1		Depositors:	BA Pulaski
Biosafety Level:	1		Shipped:	frozen
Medium & Serum:	See Propagation		Growth Properties:	adherent
Organism:	<i>Mus musculus</i> (mouse)		Morphology:	epithelial

Source: **Organ:** mammary gland
Strain: BALB/cfC3H
Disease: tumor

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.

[Related Cell Culture Products](#)

Tumorigenic:	YES
Comments:	<p>4T1 is a 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment. [49690]</p> <p>When injected into BALB/c mice, 4T1 spontaneously produces highly metastatic tumors that can metastasize to the lung, liver, lymph nodes and brain while the primary tumor is growing in situ. [49688] [49690]</p> <p>The primary tumor does not have to be removed to induce metastatic growth. The tumor growth and metastatic spread of 4T1 cells in BALB/c mice very closely mimic human breast cancer. This tumor is an animal model for stage IV human breast cancer. [49688] [49689]</p> <p>4T1-induced tumors can be used as a post-operative model as well as a non-surgical model because the 4T1-induced tumor metastasizes spontaneously in both models with similar kinetics. [49687] [49688] [49689]</p> <p>Because 4T1 is resistant to 6-thioguanine, micro-metastatic cells (as few as 1) can be detected in many distant site organs with better accuracy than most tumor models. There is no need to count nodules or weight target organs. [49687] [49688] [49689]</p>
Propagation:	<p>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%.</p> <p>Temperature: 37.0°C</p>
Subculturing:	<p>Protocol: NOTE: the cells should not be allowed to become confluent, subculture at 80% of confluence. Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.</p> <p>Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:8 is recommended</p> <p>Medium Renewal: Every 2 to 3 days</p>
Preservation:	<p>Freeze medium: Complete growth medium 95%; DMSO, 5%</p> <p>Storage temperature: liquid nitrogen vapor temperature</p>

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): [ATCC 30-2001](#)
recommended serum: [ATCC 30-2020](#)

References: 49687: Pulaski BA, et al. Immunotherapy with vaccines combining MHC class II/CD80+ tumor cells with interleukin-12 reduces established metastatic disease and stimulates immune effectors and monokine induced by interferon gamma. *Cancer Immunol. Immunother.* 49: 34-45, 2000. PubMed: [10782864](#)
49688: Pulaski BA, Ostrand-Rosenberg S. Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. *Cancer Res.* 58: 1486-1493, 1998. PubMed: [9537252](#)
49689: Pulaski BA, et al. Cooperativity of Staphylococcal aureus enterotoxin B superantigen, major histocompatibility complex class II, and CD80 for immunotherapy of advanced spontaneous metastases in a clinically relevant postoperative mouse breast cancer model. *Cancer Res.* 60: 2710-2715, 2000. PubMed: [10825145](#)
49690: Aslakson CJ, Miller FR. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. *Cancer Res.* 52: 1399-1405, 1992. PubMed: [1540948](#)

ATCC® Number:	CRL-6475™	Order this item	Price:	\$264.00
Designations:	B16-F10		Shipped:	frozen
Biosafety Level:	1		Growth Properties:	adherent
Medium & Serum:	See Propagation		Morphology:	
Organism:	<i>Mus musculus</i> (mouse)			

Source: **Organ:** skin
Strain: C57BL/6J
Disease: melanoma

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.

[Related Cell Culture Products](#)

Applications: transfection host ([technology from amaxa](#))

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

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.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:10 is recommended

Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products: recommended serum: ATCC [30-2020](#)
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC [30-2002](#)

References: 22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. *Cancer Res.* 35: 218-224, 1975. PubMed: [1109790](#)
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. *Cancer Res.* 36: 3608-3615, 1976. PubMed: [953987](#)
22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. *Cancer Res.* 37: 3945-3956, 1977. PubMed: [908034](#)
22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. *Science* 197: 893-895, 1977. PubMed: [887927](#)
23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. *J. Natl. Cancer Inst.* 60: 1217-1222, 1978. PubMed: [418183](#)
23362: . . *Nat. New Biol.* 242: 148-149, 1973.

ATCC® Number:	TIB-64™	Order this Item	Price:	\$268.00
Designations:	P815		Depositors:	P Ralph
Biosafety Level:	1		Shipped:	frozen
Medium & Serum:	See Propagation		Growth Properties:	suspension (some adherent cells)
Organism:	<i>Mus musculus</i> (mouse)		Morphology:	

Source: **Disease:** mastocytoma
Strain: DBA/2
Cell Type: mast cell;

Cellular Products: lysozyme [[1080](#)]

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.

[Related Cell Culture Products](#)

Applications: transfection host([technology from amaxa](#))

Comments:	P815 cells phagocytose latex beads but not zymosan or BCG. They do not function in antibody dependent cell mediated cytotoxicity. Growth of the cells is not inhibited by dextran sulfate, LPS or PPD. [1136] [2104] Tested and found negative for ectromelia virus (mousepox).
Propagation:	ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Temperature: 37.0°C
Subculturing:	Medium Renewal: Every 2 to 3 days Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 cells/ml and maintain between 1 X 10 exp5 and 1 X 10 exp6 cells/ml. Adherent cells can be recovered by scraping.
Preservation:	culture medium 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2002 recommended serum: ATCC 30-2020
References:	1080: Ralph P, et al. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. J. Exp. Med. 143: 1528-1533, 1976. PubMed: 1083890 1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031 1136: Ralph P, Nakoinz I. Direct toxic effects of immunopotentiators on monocytic myelomonocytic, and histiocytic or macrophage tumor cells in culture. Cancer Res. 37: 546-550, 1977. PubMed: 318922 1137: Ralph P, Nakoinz I. Lipopolysaccharides inhibit lymphosarcoma cells of bone marrow origin. Nature 249: 49-51, 1974. PubMed: 4208429 2104: Ralph P, et al. Lymphosarcoma cell growth is selectively inhibited by B lymphocyte mitogens: LPS, dextran sulfate and PPD. Biochem. Biophys. Res. Commun. 61: 1268-1275, 1974. PubMed: 4616699 22262: Lundak RL, Raidt DJ. Cellular immune response against tumor cells. I. In vitro immunization of allogeneic and syngeneic mouse spleen cell suspensions against DBA mastocytoma cells. Cell. Immunol. 9: 60-66, 1973. PubMed: 4270287 22825: Plaut M, et al. Studies on the mechanism of lymphocyte-mediated cytotoxicity. IV. Specificity of the histamine receptor on effector T cells. J. Immunol. 111: 389-394, 1973. PubMed: 4123976 29033: Schmidt W, et al. Cell-free tumor antigen peptide-based cancer vaccines. Proc. Natl. Acad. Sci. USA 94: 3262-3267, 1997. PubMed: 9096381 32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. J. Virol. 70: 7921-7928, 1996. PubMed: 8892915