

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: August 12, 2011
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards 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/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	Xiufen Zheng
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PHONE NUMBER:	519-6858500x36566
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Location of experimental work to be carried out :

Building : <u>LHSC-UC</u>	Room(s): <u>C4-230</u>
Building : <u>LHSC-UC</u>	Room(s): <u>C9-119</u>
Building : _____	Room(s): _____

***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 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: CIHR/KFOC/CBCF

GRANT TITLE(S): A new therapy for cancer

UNDERGRADUATE COURSE NAME(IF APPLICABLE): _____

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Di Chen	dchen97@uwo.ca	May16,2009
_____	_____	_____
_____	_____	_____
_____	_____	_____

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Tumour cell line (4T1, B16F10, MDA-MB-231, Renca, and RCL1932) : tumour cells will be stored at a locked -80 C freezer using capped vials. Tumour cells will be bleached with 10% bleach for at least 10 minutes before being dumped into sink.

Changes to this page

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

Cancer is the second leading cause of death in the world. Chemotherapy is the most commonly used treatment for cancer patients. However, most of the anticancer agents currently used have deleterious side effects due to little or no selectivity against normal mammalian cells. In addition, current cancer treatments give rise to multidrug resistance which greatly reduces their therapeutic usefulness. Clearly, novel therapeutic approaches with reduced risk of drug resistance development and higher cancer-selectivity are urgently required. Cationic antimicrobial peptides (AMPs) are a natural-source drug produced by many organisms as a first line of defense to fight antimicrobial invaders. Some of AMPs exhibit selective anticancer activities. However, the therapeutic effects of AMPs have not been completely investigated. In addition, how AMPs kill cancer cells is not fully understood. The objective of this proposed study is to develop a novel AMP- based therapy for breast cancer and obtain insights into the mechanisms by which AMPs selectively target cancer cells through a series of in vitro cell culture and in vivo animal (mouse model) study.

First, we will culture tumour cells in vitro and treat them with different concentrations of AMP. We will examine tumour cell death and the expression of cell death associated genes. Non-tumour cells will be included as control. This experiment will answer the questions whether AMP specifically induce tumour cell death, but not injury other non-tumour cells, and the mechanism by which AMP kills tumour cells.

Second, we will inject tumour cells into mice based on our animal protocol to induce tumour formation and growth in vivo. We will then treat tumour bearing mice with different dose of AMP with different frequencies. To assess AMP therapeutic effects, we will measure tumour size and tumor growth. At the endpoint of experiments, we will collect tumor and weigh them. We will also assess AMP's effect of metastasis. We will collect lungs, lymph nodes, livers and spleens from treatment group and control group and subsequently subjected to histochemistry staining to detect metastasis. The therapeutic effects will be compared with control group.

This study will provide a treatment for cancer with less toxicity and deepen insight into the mechanism of AMP selectively killing tumour cells.

Changes to Summary

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other 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:

Full Scientific Name of Biological Agent(s)* (Be specific)	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
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

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	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	MDA-DB231, CRL1932	1	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	4T1, B16F10, renca	1	ATCC
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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 2+ 3

Additional Comments: _____

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 Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

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

** Please attach a plasmid map.

***No Material Safety Data Sheet is required for the following strains of *E. coli*:

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

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

* Please attach a Material Safety Data Sheet or equivalent.

4.3.1 Will virus be replication defective? YES NO

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

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? 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

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? YES, number: NO PENDING

7.0 Animal Experiments

7.1 Will live animals be used? YES NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used **C57BL/6, B6, nude mice**

7.3 AUS protocol # **2011-59**

7.4 Will any of the agents listed in section 4.0 be used in live animals
 NO YES, specify:

7.5 Will the agent(s) be shed by the animal:
 YES NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES NO - If NO, please proceed to section 9.0

8.2 Will live animals be used? YES NO

8.3 If YES, please specify the animal(s) used:

- | | | |
|-----------------------------|--|--|
| ◆ Pound source dogs | <input type="checkbox"/> YES | <input checked="" type="checkbox"/> NO |
| ◆ Pound source cats | <input type="checkbox"/> YES | <input checked="" type="checkbox"/> NO |
| ◆ Cattle, sheep or goats | <input type="checkbox"/> YES, species | <input checked="" type="checkbox"/> NO |
| ◆ Non-human primates | <input type="checkbox"/> YES, species | <input checked="" type="checkbox"/> NO |
| ◆ Wild caught animals | <input type="checkbox"/> YES, species & colony # | <input checked="" type="checkbox"/> NO |
| ◆ Birds | <input type="checkbox"/> YES, species | <input checked="" type="checkbox"/> NO |
| ◆ Others (wild or domestic) | <input type="checkbox"/> YES, specify | <input checked="" type="checkbox"/> NO |

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES NO If **NO**, please proceed to Section 10.0

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

9.3 What is the LD₅₀ (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? YES NO
If **YES**, Please provide details:

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

Additional Comments: n/a

10.0 Insects

10.1 Do you use insects? 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 insect?

10.4 What is the life stage of the insect?

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

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

10.7 Do you use insects that require a permit from the CFIA permit? YES NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

- 11.1 Do you use plants? YES NO - If NO, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention? Grow and maintain a crop "One-time" use
- 11.6 Do you do any modifications to the plant? YES NO
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

- 12.1 Will any of the above agents be imported? YES, country of origin NO
If NO, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO
- 12.4 Has the import permit been sent to OHS? YES, please provide permit # NO

13.0 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 biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement...
Enter Your Name Xiufen Zheng **Date:** Oct 7,2011

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
 YES, location and date of most recent biosafety inspection: **LHSC, C4-200**
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? YES NO
If YES please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
In the event of an exposure the incident should be reported to Occupational Health rather than the Biosafety Committee.

15.3 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.shs.uwo.ca/workplace/newposition.htm>

An X in the check box indicates you agree with the above statement...
Enter Your Name Xiufen Zheng Date: Oct.7, 2011

15.4 Additional Comments: _____

Changes to 15.2

16.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: DEC 13, 2012

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

Special Conditions of Approval:

Cell Line Designation: 4T1
ATCC® Catalog No. CRL-2539™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Mus musculus* (mouse)

Strain: BALB/cfC3H

Tissue: tumor; breast; mammary gland

Morphology: epithelial

Growth properties: adherent

Tumorigenic: Yes, forms tumors and metastasizes in BALB/c mice.

Depositor: B.A. Pulaski

Comments: 4T1 is a 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment [PubMed: 1540948]. 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* [PubMed: 9537252; PubMed: 1540948]. 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. [PubMed: 9537252; PubMed: 10825145]. 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. [PubMed 10782864; PubMed: 9537252; PubMed: 10825145].

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. [PubMed 10782864; PubMed: 9537252; PubMed: 10825145].

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bml4/bml4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 10 minutes.
4. Resuspend the cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted



Product Information Sheet for ATCC® CRL-2539™

microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

NOTE: the cells should not be allowed to become confluent, subculture at 80% of confluence.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which 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.
Subcultivation Ratio: 1:6 to 1:8.
6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Every 2 to 3 days

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%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) and ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

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

Pulaski BA and 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

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

Aslakson CJ and 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

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines.** 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture.** *J. Tissue Culture Methods* 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

American Type Culture Collection
P.O. Box 1548
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
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Cell Line Designation: B16-F10
ATCC[®] Catalog No. CRL-6475[™]

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Cell Line Description

Organism: *Mus musculus* (mouse)

Strain: C57BL/6J

Tissue: melanoma; skin

Growth properties: adherent

Morphology: mixture of spindle-shaped and epithelial-like cells

Depositors: Naval Biosciences Laboratory

Comments: Confirmed as a murine cell line by ATCC.

Tests for microbial contamination were negative.

DISCLAIMER: This cell line was deposited by the Naval Biosciences Laboratory. Researchers should be aware that one purpose of the NBL bank was to produce and distribute early passage cultures of cells from various clinical materials. Many of these lines, unlike most others available and published in the ATCC catalog, are primary and mixed. Those from tumors, for example, may consist of mixtures of stromal and cancer cells in which the former cell types predominate.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmb14/bmb14toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately $125 \times g$ for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. *It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells, grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes



Product Information Sheet for ATCC® CRL-6475™

handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. If the cells are still attached, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which 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.
Subculture ratio: 1:6 to 1:10
6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in *Culture Of Animal Cells: A Manual Of Basic Technique* by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Medium Renewal

Every 2 to 3 days

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%

This medium is formulated for use with a 5% CO₂ in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO₂ in air atmosphere is then recommended).

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) and ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(Additional references may be available in the catalog description at www.atcc.org)

Fidler IJ. **Biological behavior of malignant melanoma cells correlated to their survival in vivo.** *Cancer Res.* 35: 218-224, 1975 PubMed: 75074100

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: 76255481

Fidler IJ and Bucana C. **Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes.** *Cancer Res.* 37: 3945-3956, 1977 PubMed: 78001804

Fidler IJ and Kripke ML. **Metastasis results from preexisting variant cells within a malignant tumor.** *Science* 197: 893-895, 1977 PubMed: 77236031

Briles EB and Kornfeld S. **Isolation and metastatic properties of detachment variants of B16 melanoma cells.** *J. Natl. Cancer Inst.* 60: 1217-1222, 1978 PubMed: 78173966

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Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

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Cell Line Designation: 786-O
ATCC® Catalog No. CRL-1932

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- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)

Tissue: renal cell adenocarcinoma; kidney

Age: 58 years

Gender: male

Ethnicity: Caucasian

Morphology: epithelial

Growth properties: adherent

Tumorigenic: yes, in immunosuppressed hamsters

Karyotype: hypertriploid; Y was present in 60% the cells examined

DNA profile (STR analysis)

Amelogenin: X,Y

CSF1PO: 10

D13S317: 8

D16S539: 12

D5S818: 9

D7S820: 11,12

TH01: 6,9,3

TPOX: 8,11

vWA: 15,17

Depositors: R.D. Williams

Comments: This line was derived from a primary clear cell adenocarcinoma. The cells display both microvilli and desmosomes, and can be grown in soft agar.

The cells produce a PTH like peptides that is identical to peptides produced by breast and lung tumors. The peptide has an N terminal sequence similar to PTH, has PTH like activity, and has a molecular weight of 6000 daltons.

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. 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. 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*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a 75 cm^2 tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

4. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which 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.
Split Ratio: 1:4 to 1:12

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly

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%

This medium is formulated for use with a 5% CO₂ in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Williams RD et al. *In vitro* cultivation of human renal cell cancer. I. Establishment of cells in culture. *In Vitro* 12: 623-627, 1976 PubMed: 77092774

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Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), *ATCC Quality Control Methods for Cell Lines*. 2nd edition, Published by ATCC.

Caputo, J. L., *Biosafety procedures in cell culture*. *J. Tissue Culture Methods* 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) *Laboratory Safety: Principles and Practice*. Second edition, ASM press, Washington, DC.

Cell Line Designation: MDA-MB-231**ATCC® Catalog No. HTB-26™****Table of Contents:**

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- Cryoprotectant Medium
- References
- Warranty

Cell Line Description**Organism:** *Homo sapiens* (human)**Tissue:** mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion**Age:** 51 years**Gender:** female**Ethnicity:** Caucasian**Tumorigenic:** yes, in nude mice also in ALS treated BALB/c mice; forms poorly differentiated adenocarcinoma (grade III)**AntigenExp:** Blood Type O; Rh-**DNA profile (STR analysis):**

Amelogenin: X
 CSF1PO: 12,13
 D13S317: 13
 D16S539: 12
 D5S818: 12
 D7S820: 8,9
 TH01: 7,9,3
 TPOX: 8,9
 vWA: 15,18

Morphology: epithelial**Growth properties:** adherent**Receptors expressed:** epidermal growth factor (EGF); transforming growth factor alpha (TGF alpha)**Depositors:** R. Cailleau**Comments:** The cells express the WNT7B oncogene [PubMed: 8168088].

Karyology: The cell line is aneuploid female, with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al., *Cancer Genet. Cytogenet.* 3: 61, 1981.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA: from this line is available as ATCC Catalog HTB-26D™ (10µg)

Total RNA from this line is available as ATCC HTB-26R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this*

point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 xg for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a new culture flask.
5. Incubate the culture at 37°C in a suitable incubator. (without CO₂)

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. If the cells are still attached, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a free gas exchange with atmospheric air until they are ready to be subcultured.
3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37° in a free gas exchange with atmospheric air until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum, which 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 with 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 cell suspension to new culture vessels.
Subcultivation Ratio: 1:2 to 1:4.
6. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

2 to 3 times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO₂ and air mixture is detrimental to cells when using this medium for cultivation

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references are available in the catalog at www.atcc.org)

Brinkley BR et al. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. *Cancer Res.* 40: 3118-3129, 1980 PubMed: 81042058

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Product Information Sheet for ATCC HTB-26™

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Caputo, J. L., **Biosafety procedures in cell culture.** *J. Tissue Culture Methods* 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tullis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

ATCC Warranty

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Cell Biology

ATCC® Number: **CRL-2947™** Order this Item Price: **\$379.00**

Designations: **Renca**
Depositors: R. Wiltrout
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)

Morphology: epithelial-like



Source: **Organ:** kidney
Disease: renal adenocarcinoma
Cell Type: epithelial
Strain: Balb/cCr

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.

Isolation: Isolation date: 1969
Tumorigenic: YES
Age: 6 weeks
Gender: male

Comments: The Renca cell line was derived from a tumor that arose spontaneously as a renal cortical adenocarcinoma in Balb/cCr mice. The pattern of growth of this tumor accurately mimics that of human adult renal cell carcinoma, particularly with regard to spontaneous metastasis to lung and liver. [PubMed: 4703766, 4057425]The cells do not express transforming growth factor-beta type II receptor (TbetaR-II). [PubMed: 10414746]

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:

- 10% fetal bovine serum (final conc.)
- non-essential amino acids (NEAA) (0.1mM extra)
- additional sodium pyruvate (1mM extra)
- additional L-glutamine (2mM extra)

Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

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Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Subculturing:
1. Remove and discard culture medium.
 2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which 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 37C 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⁽⁴⁾ to 4 X 10⁽⁴⁾ viable cells/sq. cm is recommended.
 6. Incubate cultures at 37C. We recommend that you subculture when the culture reaches a cell concentration between 8 X 10⁽⁴⁾ and 1.5 X 10⁽⁵⁾ cells/sq. cm.
Subcultivation ratio: A subcultivation ratio of 1:4 to 1:10 is recommended.
Medium renewal: Every 2 to 3 days

Preservation: **Freeze medium:** RPMI-1640 Medium, 77.5%; FBS, 15% FBS; DMSO, 7.5%

Doubling Time: **Storage temperature** liquid nitrogen vapor phase
approximately 24 hours

Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2001

Recommended serum: ATCC 30-30-2020
0.25% (w/v) Trypsin - 0.53mM EDTA in Hank's BSS (w/o

Related Products: Ca⁺⁺, Mg ⁺⁺): ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X
L-Glutamine solution, 200mM: ATCC 30-2214
Erythrosin B vital stain solution: ATCC 30-2404