

**The University of Western Ontario**  
**BIOLOGICAL AGENTS REGISTRY FORM**  
 Approved Biohazards Subcommittee: October 14, 2011  
 Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://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, 1<sup>st</sup> 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](mailto: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](mailto: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/](http://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:	<b>Dr. Charles McKenzie</b>
DEPARTMENT:	<b>Medical Biophysics</b>
ADDRESS:	<b>Natural Sciences Building, Room 9</b>
PHONE NUMBER:	<b>(519) 661-2111 x85686</b>
EMERGENCY PHONE NUMBER(S):	<b>(519) 614-8145</b>
EMAIL:	<b>cmcken@uwo.ca</b>

Location of experimental work to be carried out :

Building :	<b>Robarts Research Institute</b>	Room(s):	<b>2276, 2245-B, 2245-E</b>
Building :		Room(s):	
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: **Ontario Research Fund**

GRANT TITLE(S): **Ontario Preclinical Imaging Consortium**

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>
Patrick Lim	<a href="mailto:hlim62@uwo.ca">hlim62@uwo.ca</a>	23/11/11
Paula Pflugfelder	<a href="mailto:pflugfe@imaging.robarts.ca">pflugfe@imaging.robarts.ca</a>	11/1/12



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

**The purpose of this project is to develop and test magnetic resonance imaging (MRI) techniques for seeing the abnormal function of cancer cells in breast and brain tumors.**

**Normal cells and cancer cells can process different molecules in different ways. The development of a method to monitor how tumors process molecules will allow diagnosis of cancers and could be useful for monitoring the response of tumors to therapy. MRI is a procedure that uses magnetic waves to take a picture of various parts of the body. Unlike many other imaging methods, radiation is not used in MRI making it much safer than other methods.**

**The purpose of this research is to develop and test methods that will allow monitoring of the processing of molecules that are specially modified to make them visible in an MR image. The technique we are developing relies on seeing changes in the molecules we inject that occur in living animals, so its application requires validation in an animal tumor model. There are no other radiation free methods that allow imaging of these cellular processes.**

## 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](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-MB-231 (ATCC #HTB-26)	Level 1	NCI through Foster lab
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	C6 Glioblastoma (ATCC #CCL-107)	Level 1	Cedarlane through Lee Labs
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](http://www.atcc.org))

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required  1  2  2+  3

Additional Comments: **We will not be culturing the cells, we will obtain them from other labs and use them for implantation only.**

## 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](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 7.0

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 **Rat, Mouse**

7.3 AUS protocol # **2011-087**

7.4 List the location(s) for the animal experimentation and housing. **HSACF ,RRI 2276, RRI 2245-B, 2245-E**

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

*7.5 -> No agents listed in 4.0*

7.6 Will the agent(s) be shed by the animal:  
 YES  NO, please justify: **Cells are implanted in rat brain or mouse fat pad, and cannot be shed after surgery.**

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

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

## 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

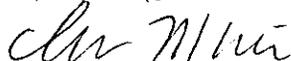
All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ 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 Charles Date: 2012-02-01

McKenzie  


**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:
- 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 accidental splash, the liquid containing these cells will be washed off with soap and water. In the case of a needlestick injury, the wound will be expressed to bleed, washed with soap and water and bandaged. The individual will be directed to go to UWO staff health.**

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/workplacehealth.html>

*An X in the check box indicates you agree with the above statement...*

*Enter Your Name Charles Date: 2012-02-01*

*McKenzie Clu Min*

15.4 Additional Comments: \_\_\_\_\_

**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: Ronald Abbott  
Date: Feb. 06, 2012

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:

**Cell Line Designation: MDA-MB-231****ATCC® Catalog No. HTB-26™****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
- 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/bmbi4/bmbi4toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbi4/bmbi4toc.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 CO<sub>2</sub>)

### 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 cm<sup>2</sup> 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 cm<sup>2</sup> 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 CO<sub>2</sub> 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](http://www.atcc.org), or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

### References

(additional references are available in the catalog at [www.atcc.org](http://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

Cruciger Q et al. **Morphological, biochemical and chromosomal characterization of breast tumor lines from pleural effusions.** *In Vitro* 12: 331, 1976

Siciliano MJ et al. **Mutually exclusive genetic signatures of human breast tumor cell lines with a**

**common chromosomal marker.** *Cancer Res.* 39: 919-922, 1979 PubMed: 79146816

Cailleau R et al. **Breast tumor cell lines from pleural effusions.** *J. Natl. Cancer Inst.* 53: 661-674, 1974 PubMed: 75007579

Cailleau R et al. **Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization.** *In Vitro* 14: 911-915, 1978 PubMed: 79087497

Bates SE et al. **Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells.** *Endocrinology* 126: 596-607, 1990 PubMed: 90092004

Dickstein B et al. **Increased epidermal growth factor receptor in an estrogen-responsive, adriamycin-resistant MCF-7 cell line.** *J. Cell. Physiol.* 157: 110-118, 1993 PubMed: 94012995

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue.** *Cancer Res.* 54: 2615-2621, 1994 PubMed: 94221588

Satya-Prakash KL et al. **Cytogenetic analysis on eight human breast tumor cell lines: high frequencies of 1q, 11q and HeLa-like marker chromosomes.** *Cancer Genet. Cytogenet.* 3: 61-73, 1981 PubMed: 82001960

Katayose Y et al. **Promoting apoptosis: a novel activity associated with the Cyclin-dependent kinase inhibitor p27.** *Cancer Res.* 57: 5441-5445, 1997 PubMed: 98069835

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma.** *Cancer Res.* 57: 5386-5390, 1997 PubMed: 98053913

Sheng S et al. **Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells.** *Proc. Natl. Acad. Sci. USA* 93: 11669-11674, 1996 PubMed: 97030253

De Vincenzo R et al. **Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines.** *Anti-Cancer Drug Des.* 13: 19-33, 1998 PubMed: 98134515

Soker S et al. **Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-encoded domain.** *J. Biol. Chem.* 271: 5761-5767, 1996 PubMed: 96215040

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines.** 2<sup>nd</sup> 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 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

### Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org).

© ATCC 2007. All rights reserved.

ATCC® is a registered trademark of the American Type Culture Collection. 07/07

## Cell Line Designation: C6 ATCC Catalog No. CCL-107<sup>™</sup>

### 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:** *Rattus norvegicus* (rat)

**Tissue:** glioma; brain; glial cell

**Morphology:** fibroblast

**Growth properties:** adherent

**Virus Susceptibility:** vaccinia; vesicular stomatitis (Indiana); herpes simplex

**Virus Resistance:** poliovirus 3

**Reverse Transcriptase:** negative

**Products:** S-100 protein; somatotrophin; cells produce glyceryl phosphate dehydrogenase in response to glucocorticoids

**Receptors Expressed:** glucocorticoid

**Depositor:** G. Sato

**Comments:** The glial cell strain, C6, was cloned from a rat glial tumor induced by N-nitrosomethylurea by Benda et al. after a series of alternate culture and animal passages. S-100 production increases ten fold as cells grow from low density to confluency.

**Karyology:** Stemline number is diploid. Karyotype is stable within the stemline number and is that of a normal male. Three cells with breaks; one with a secondary constriction, one with a dicentric, one with a rearrangement and four with terminal or centromere associations.

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

### 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](http://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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $25 \text{ cm}^2$  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  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  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 (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% CO<sub>2</sub> 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 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

Volumes used in this protocol are for a 75 cm<sup>2</sup> 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 - 0.53 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 10 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:** A subcultivation ratio of 1:2 to 1:3 is recommended.

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**

Two to three times per week

**Complete Growth Medium**

The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 2.5%
- horse serum to a final concentration of 15%.

This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere.

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

ATCC tested horse serum is available as ATCC® Catalog No. 30-2040 (500ml) and ATCC® Catalog No. 30-2041 (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](http://www.atcc.org), or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

**References**

(Additional references may be available in the catalog description at [www.atcc.org](http://www.atcc.org).)

Benda, P., et al. **Differentiated rat glial cell strain in tissue culture**. *Science* 161: 370-371, 1968 PubMed: 68322597

Lightbody, J.J., et al. **Establishment of differentiated clonal strains of glial brain cells in culture**. *Fed. Proc.* 27: 720, 1968.

Chen, Y., et al. **Demonstration of binding of dengue virus envelope protein to target cells**. *J. Virol.* 70: 8765-8772, 1996 PubMed: 97126081.

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2<sup>nd</sup> 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](mailto:tech@atcc.org). Or you may contact your local distributor.

**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org).

© ATCC 2007. All rights reserved.  
ATCC® is a registered trademark of the American Type Culture Collection.  
07/07