

# Modification Form for Permit BIO-RRI-0023

Permit Holder: Arthur Brown

## Approved Personnel

(Please stroke out any personnel to be removed)

Adam Verhoeve  
Anna Pniak  
Kathy Xu  
Nicole Geremia

## Additional Personnel

(Please list additional personnel here)

William Monty Mckillop  
Magdalena Dragan

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli	
Approved Cells	Human (primary), Rodent (primary and established), A7, Neu 7, DI-TNC1, HEK 293	Normal Human Astrocytes from Lonza
Approved Use of Human Source Material	Bone marrow	
Approved GMO	SV 40 Large T antigen, PGL 3, PGL4, pCMV, E1A oncogene	
Approved use of Animals	Mice (w spinal cord injury)	

\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

\*\* PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 8, 2007

Signature of Permit Holder: Arthur Brown

BioSafety Officer(s): \_\_\_\_\_

Chair, Biohazards Subcommittee: \_\_\_\_\_

**Modification Form for Permit BIO-RRI-0023**

**Permit Holder: Arthur Brown**

Approved Toxin(s)

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These cells will be cultured with various media. The cells will be lysed and their RNA or protein harvested. The levels of different mRNAs will be assayed by quantitative PCR and the levels of proteins by immunocytochemistry or Western Blot analysis.

\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

\*\* PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification:   2  

Date of last Biohazardous Agents Registry Form:   Jan 8, 2007  

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BioSafety Officer(s): \_\_\_\_\_

Chair, Biohazards Subcommittee: \_\_\_\_\_

## Clonetics<sup>®</sup> Normal Human Astrocytes

### Instructions for Use

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**Receiving Instructions:** Unpack immediately! Packages may contain components with various storage requirements!

#### Safety

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

**WARNING: CLONETICS<sup>®</sup> AND POIETICS<sup>®</sup> PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Scientific Support.

#### Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. For proliferating cells – swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO<sub>2</sub>, humidified incubator and allow to equilibrate for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.
4. AGM<sup>™</sup> BulletKit<sup>®</sup> Instructions: Upon arrival, store Astrocyte Basal Medium (ABM<sup>™</sup>) at 2°C to 8°C and SingleQuots<sup>®</sup> at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2°C to 8°C and added to ABM<sup>™</sup> within 72 hours of receipt. After SingleQuots<sup>®</sup> are added to basal medium, use within one month. Do not re-freeze.

5. ReagentPack<sup>™</sup> Subculture Reagents are sterile-filtered and then stored at -20°C until shipment. Subculture reagents may thaw during transport. They may be refrozen once. If you plan to use within 3 days, store at 2°C to 8°C. Trypsin/ EDTA Solution has a limited shelf life or activation at 2°C to 8°C. If, upon arrival, Trypsin/EDTA is thawed, immediately aliquot and refreeze at -20°C. We recommend that the HEPES-BSS and the Trypsin Neutralizing Solution be stored at 2° to 8°C for no longer than one month.

**Note:** To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at -20°C.

Using media or reagents other than what's recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

#### Preparation of Media

**For a BulletKit<sup>®</sup>, perform the following steps:**

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label, allowing for the basal medium lot number and expiration date) to be visible.
5. Record the new expiration date on the label based on the shelf life.

**Note:** If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2 µm filter to assure sterility. Routine refiltration is not recommended.

## Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density for NHA is 5,000 cells/cm<sup>2</sup>.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density of 5,000 cells/cm<sup>2</sup> and the surface area of the vessels being used. Do not seed cells into a well plate immediately out of cryopreservation. Add the appropriate amount of medium to the vessels (1 ml/5 cm<sup>2</sup>) and allow the vessels to equilibrate in a 37°C, 5% CO<sub>2</sub>, humidified incubator for at least 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath by gently swirling the vial only until the ice disappears. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
4. Resuspend the cells in the cryovial and dispense them into the preincubated culture vessels at the calculated seeding density. Gently rock the culture vessel to evenly distribute the cells and return to the incubator.
5. Centrifugation should not be used to remove cells from cryopreservation medium. This action is more damaging than the effects of residual DMSO in the culture.

## Subculturing

The following instructions are for a 25 cm<sup>2</sup> flask. Adjust all volumes accordingly for other size flasks.

### Preparation for subculturing the first flask:

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures throughout the flask.
2. For each 25 cm<sup>2</sup> of cells to be subcultured:
  - Thaw 2 ml of Trypsin/EDTA and allow to come to room temperature.
  - Allow 7-10 ml of HEPES Buffered Saline Solution (HEPES-BSS) to come to room temperature.

- Allow 4 ml of Trypsin Neutralizing Solution (TNS) to come to room temperature.
3. Remove growth medium from 2°C to 8°C storage and allow to warm to room temperature.
  4. Prepare new culture flasks by adding pre-warmed medium at a volume of 1 ml/5 cm<sup>2</sup> of surface area, label with cell strain, lot number, passage and date. Place the flasks in the incubator until cells are ready to be seeded.

### In a sterile field:

1. Aspirate the medium from one flask.
2. Rinse the cells with 5 ml of room temperature HEPES-BSS. DO NOT forget this step. The medium contains trypsin inhibitors such as calcium or serum proteins.
3. Aspirate the HEPES-BSS from the flask.
4. Cover the cells with 2 ml of Trypsin/EDTA solution.
5. Place the flask in the 37°C incubator for 3 to 4 minutes.
6. Allow the trypsinization to continue until approximately 90% of the cells have rounded up. Examine the culture flask under the microscope to determine the extent of the detachment.
7. At this point, rap the edge of the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, return the flask to the incubator for 30 seconds to 1 minute.
8. After the cells have detached, neutralize the trypsin in the flask with 4 ml of room temperature Trypsin Neutralizing Solution.
9. If a majority of the cells do not detach within 5 minutes, the enzyme activity of the trypsin has been compromised by low temperature or an overextended shelf life. Proceed to harvest the cells from the flask as described above, and either re-trypsinize with fresh, warm Trypsin/EDTA solution or rinse with Trypsin Neutralizing Solution, add fresh, warm medium to the flask and return to the incubator until fresh trypsinization reagents are available.
10. Quickly transfer the detached cells to a sterile 15 ml centrifuge tube.
11. Rinse the flask with a final 2 ml of HEPES-BSS to collect residual cells, and add this rinse to the centrifuge tube.
12. Examine the flask under the microscope to make sure the harvest was successful by observing the number of cells remaining. This should be less than 5%.
13. Centrifuge the cell suspension at 160 x g to 200 x g for 5 minutes at 2°C to 8°C.

14. Aspirate the supernatant and resuspend the cell pellet in 2 ml of AGM™.
15. Determine cell count and viability using a hemacytometer and Trypan Blue.
16. Calculate the volume of the cell suspension needed to seed the flask at a density of 5,000 cells/cm<sup>2</sup> and add the appropriate volume of cell suspension to a tissue culture flask or dish (pre-filled at 1 ml/5cm<sup>2</sup> of surface area with pre-warmed medium). If seeding into well plates at this time, the recommended density is 10,000 cells/cm<sup>2</sup>. Mix gently to evenly distribute the cells and return the flask to the incubator.

## Maintenance

1. Change the growth medium the day after seeding and every other day thereafter. As the cells become more confluent, increase the volume of media as follows: under 25% confluence then feed cells 1 ml per 5 cm<sup>2</sup>, 25-45% confluence then feed cells 1.5 ml per 5 cm<sup>2</sup>, over 45% confluence then feed cells 2 ml per 5 cm<sup>2</sup>.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove spent medium from flask and replace it with the warmed, fresh medium and return the flask to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

## Ordering Information

<b>Cryopreserved Cells (Single donor)</b>		
CC-2565	NHA	≥1,000,000 cells
<b>Proliferating Cells</b>		
CC-2665	NHA	T-25 flask
CC-0297	NHA	T-75 flask
CC2565T150	NHA	T-150 flask
CC2565T225	NHA	T-225 flask
<b>Proliferating Cells in PreSeeded Plates</b>		
CC-2565W6	NHA	6 wells
CC-2565W12	NHA	12 wells
CC-2565W24	NHA	24 wells
CC-2565W48	NHA	48 wells
CC-0093	NHA	96 wells

## Related Products

### Astrocyte Medium (Must be purchased separately):

CC-3186	AGM™ BulletKit®	Kit which contains a 500 ml bottle of ABM™, (CC-3187) and AGM™ SingleQuotes® (CC-4123)
CC-3187	ABM™	Astrocyte Basal Medium (no growth factors) (500 ml)
CC-4123	AGM™ SingleQuotes®	Supplements for a complete growth medium, developed especially for NHA (CC-2565)

## Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells in the following manner only if Clonetics® Media and Reagents are used.

1. Clonetics® NHA Cryopreserved Cultures are assured for experimental use for at least ten population doublings.
2. Clonetics® NHA Proliferating Cultures are assured for experimental use for at least five population doublings.
3. Additional population doublings and subcultures are possible, but growth rate, biological responsiveness and function deteriorate with subsequent passage.
4. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 80% confluence.

## Quality Control

HIV-1, Hepatitis B and Hepatitis C are not detected for all donors and/or cell lots. Routine characterization of NHA includes positive immunofluorescence staining for Glial Fibrillary Acid Protein (GFAP) in the first passage out of cryopreservation; GFAP expression decreases with passaging. For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or for technical support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or technical support you may contact Lonza by web, e-mail, telephone, fax or mail.

# Summary of Approvals for Permit BIO-RRI-0023

**Permit Holder: Arthur Brown**

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

**Additional Personnel**

Allyson Tighe  
 Adam Verhoeve  
 Anna Priak  
 Kathy Xu  
 Nicole Geremia

	Please stroke out any approved Biohazards* to be removed below	Write additional Biohazards for approval below.
Approved Microorganisms*	E. coli	
Approved Cells*	Human (primary), Rodent (primary and established), A7, Neu 7	DI-TNC1 HEK 293
Approved Use of Human Source Material*	Bone marrow	
Approved GMO*	SV 40 Large T antigen, PGL 3, PGL4, pCMV	ETA oncogene <i>al.</i>
Approved use of Animals*	Mice (w spinal cord injury)	
Approved Toxin(s)*		

Date of last Biohazardous Agents Registry Form Jan 8, 2007

Signature of Permit Holder: Arthur Brown

BioSafety Officer(s): A. Stanley March 28/08

Chair, Biohazards Subcommittee: G. M. Kidder  
 4 April 2008



BIO-PR-0023

BIOHAZARDOUS AGENTS REGISTRY FORM

Reviewed by Biosafety Subcommittee: February 2006

This form must be completed by each Principal Investigator when completing a grant application or grant renewal to be administered by the Robarts Research Institute, if the use of biohazardous and/or infectious agents is proposed. For any proposed animal work involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans, this form must also be completed.

COMPLETED FORMS ARE TO BE RETURNED TO BIOSAFETY SUBCOMMITTEE CHAIR, ROOM 3-34.1.

If there are any changes to the information on these forms (excluding grant title and funding agencies) a new form must be completed and sent to the Biosafety Subcommittee Chair BEFORE implementation of these changes can occur.

If multi-team grants are being applied for, each individual Investigator of the team must submit a Biohazardous Agents Registry Form to the Biosafety Subcommittee Chair.

Containment Levels will be required in accordance with Health Canada (HC), Laboratory Biosafety Guidelines, 3rd edition 2004, or Canadian Food Inspection Agency (CFIA), Containment Standards for Veterinary Facilities, 1st edition 1998.

For questions regarding this form, please contact Biosafety Subcommittee Chair at ext. 34125.

1.0 Contact Information

PRINCIPAL INVESTIGATOR: Dr Arthur Brown
SIGNATURE: Arthur Brown
DATE: Jan 2 107
DEPARTMENT: BTRG
ADDRESS:
TELEPHONE: x 34308
EMAIL: abrown@robarts.ca

Location of experimental work to be carried out.

Building(s):
Room(s) 3-36, 211

\*For work being performed at Institutions affiliated with the Robarts Research Institute, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Robarts Research Institute, Biosafety Subcommittee Chair. See Section 13.0. Approvals

GRANT TITLE(S):

ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) EXPLAINING THE BIOHAZARD(S) USED.

FUNDING AGENCY/AGENCIES: CIHR, ORF, Heart & Stroke

Anticipated Grant End Date: \_\_\_\_\_

Names of all personnel working under Principal Investigator's supervision in this location:

All current lab personnel List names

Wicoko Geremia

Irene Ngai-Kwantha

Kathy Xue

Anna Pniak

Adam Verhoeve

Allyson Tighe

Paul Gris

Note : A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site : <http://www.phac-aspc.gc.ca/ols-bsl/index.html>

## 2.0 Microorganisms

2.1 Does your work involve the use of microorganisms? YES  NO   
If NO, please proceed to Section 3.0

2.2 Please complete the table below:

Name of Microorganism	Is microorganism a known human pathogen? YES/NO	Is microorganism a known animal pathogen? YES/NO	Is microorganism a known zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?	Health Canada or CFIA Containment Level (select one)
<u>E. coli (competent, non pathogenic)</u>	<u>No</u>	<u>No</u>	<u>No</u>	<u>1L</u>	<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
					<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
					<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

**3.0 Cell Culture**

3.1 Does your work involve the use of cell cultures? YES  NO   
 If NO, please proceed to Section 4.0.

3.2 Please indicate in the table below the type of cells that will be grown in culture.

Cell Type	Is this cell type used in your work? YES / NO	Established or Primary *	Supplier of Primary Cell Culture Tissue
Human	Yes	Primary	Dr. A. Xenocostas
Rodent	Yes	Primary & Established	generated in lab ? ←
Non-human primate	No		
Other (specify)			

established comes from J. Fawcett

\* i.e. derived from fresh tissue

3.3 Complete the following table:

Specific Cell Line	Source / Supplier	HC or CFIA Containment Level (select one)
A7	Dr. J. Fawcett	1 <input type="radio"/> 2 <input checked="" type="radio"/> 3 <input type="radio"/>
Neu7	"	1 <input type="radio"/> 2 <input checked="" type="radio"/> 3 <input type="radio"/>
		1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>

Primary genes 211.

see attached

**4.0 Use of Human Source Materials**

4.1 Does your work involve the use of human source materials? YES  NO   
 If NO, please proceed to Section 5.0

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

Human Source Material	Specify Source, or Not Applicable (NA)	Is Human Source Material known to be infected with an infectious agent? YES/NO	Name of Infectious Agent	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid	Bone marrow from healthy donors.	No		1 <input type="radio"/> 2 <input checked="" type="radio"/> 3 <input type="radio"/>
Human Blood (fraction) or other Body Fluid				1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>
Human Organs (unpreserved)				1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>
Human Tissues (unpreserved)				1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>

@this?? work-up of healthy donors before transplant (Dr. A. Xenocostas)

**5.0 Genetically Modified Organisms and Cell lines**

5.1 Will genetic modifications be made to the organism, virus or cell line? YES  NO   
If NO, please proceed to Section 6.0

5.2 Will genetic sequences from any of the following be involved?

- HIV YES  NO

If YES, specify: \_\_\_\_\_

- HTLV 1 or 2 YES  NO

If YES, specify: \_\_\_\_\_

- Other human or animal pathogen and/or their toxins YES  NO

If YES, specify: \_\_\_\_\_

5.2 Will intact genetic sequences be used from:

- SV 40 Large T antigen YES  NO
- Adeno E1A YES  NO
- Known or suspected oncogenes YES  NO

If YES, specify: A7 line carries SV40 LgT & Neu7 carries Neu  
*Oncogene.*

5.4 Will a live vector(s) (viral or bacterial) be used for gene transduction? YES  NO

If YES, name vector: ~~SV40~~ PGL3

5.5 List specific vector(s) to be used: PGL3, PGL4, pCMV

5.6 Will vector be replication defective? YES  NO

5.7 Will vector be infectious to humans or animals? YES  NO

5.8 Will this be expected to increase the Containment Level required? YES  NO

**6.0 Human Gene Therapy Trials**

6.1 Will human clinical trials using the vector(s) in 5.5 be conducted? YES  NO

If NO, please proceed to Section 7.0

If YES, attach a full description of the make-up of the virus.

6.2 Will vector be able to replicate in the host? YES  NO

6.3 How will the vector be administered? \_\_\_\_\_

6.4 Please give the Health Care Facility where the clinical trial will be conducted.  
\_\_\_\_\_  
\_\_\_\_\_

6.5 Has human ethics approval been obtained? YES  NO

Approval # \_\_\_\_\_

**7.0 Animal Experiments**

7.1 Will any of the agents listed be used in live animals? YES  NO   
If NO, please proceed to section 8.0

7.2 Name of animal species to be used: Mouse Bone Marrow-derived cells to be

7.3 AUS protocol # 2003-001-01 + transplanted into spinal cord injured mice.

7.4 If using murine cell lines, have they been tested for murine pathogens? YES  NO

**8.0 Use of Animal species with Zoonotic Hazards**

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

- Pound source dogs YES  NO
- Pound source cats YES  NO
- Sheep or goats YES  NO
- Non- Human Primates YES  NO

If YES specify species \_\_\_\_\_

- Wild caught animals YES  NO

If YES specify species \_\_\_\_\_

**9.0 Biological Toxins**

9.1 Will toxins of biological origin be used? YES  NO   
If NO, please proceed to Section 10.0  
If YES, please name the toxin \_\_\_\_\_

9.2 What is the LD<sub>50</sub> (specify species) of the toxin? \_\_\_\_\_

**10.0 Import Requirements**

10.1 Will the agent be imported? YES  NO   
If NO, please proceed to Section 11.0  
If YES, country of origin United Kingdom

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

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

10.4 Has the import permit been sent to Biosafety Subcommittee Chair? YES  NO

If YES, Permit # PHAC P-13551, CFIA-A-2006-03344-4

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In-  
progr 25  
EW

**11.0 Training Requirements for Personnel Named on Form**

All personnel named in section 1.0 of this form who will be using any of the above named agents are required to attend the following training courses given by OH&S.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 2.0 to 10.0 have been trained as required.

SIGNATURE *Allen B...*

**12.0 Containment Levels**

12.1 For the work described in sections 2.0 to 10.0, select the highest HC or CFIA Containment Level required.                      1                       2                       3

12.2 Has the facility been certified by Biosafety Subcommittee Chair for this level of containment?                      YES                       NO

If YES, give date: February 02, 2007 and permit number: 2007-01 (7-111)

**13.0 Approvals**

Roberts Research Institute

Signature *G.M. K...* Date 2 April 2008

Biosafety Officer for the Institution where experiments will take place

Signature \_\_\_\_\_ Date \_\_\_\_\_

Biosafety Officer of Roberts Research Institute (if different than above)

Signature \_\_\_\_\_ Date \_\_\_\_\_

Note: This permit will be in effect from \_\_\_\_\_ to \_\_\_\_\_  
subject to annual facility re-certification.