

# Modification Form for Permit BIO-UWO-0122

## Permit Holder: Moshmi Bhattacharya

*PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.  
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.*

**Approved Personnel**

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

Josh Burley  
Donna Cvetkovic  
~~Mistre Alemayehu~~  
Cynthia Pape  
Jeff Law

**Additional Personnel**

**(Please list additional personnel here)**

Magda Dragon

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
<b>Approved Microorganisms</b>	E. coli DH5 alpha	
<b>Approved Primary and Established Cells</b>	human (established): HEK 293, MDA-MB-231, MDA-MB-231shBarr1/2, MDA-MB-435S, MDA-MB-468, MCF-7, MCF-10A, MCF-12, SK-BR-3, Hs 578T, Hs 578 BST, OCVA429, JEG-3, JAR, HTR8/Svneo, MCF10aCL1	<div style="border: 1px solid blue; padding: 5px; color: blue;">                     mefloa pFlag A1 (vector)                      mefloa Fl-KISSIR (GPR54)                      MDA-MB-231 pFlag A1 (vector)                      MDA-MB-231 Fl-KISSIR                      MDA-MB-231 RalGDS mutant (616-768)                 </div>
<b>Approved Use of Human Source Material</b>	Human Organs or Tissues [preserved]: LLSG/UH	<div style="border: 1px solid blue; padding: 5px; color: blue;">                     MDA-MB-231 sh Ral A                      MDA-MB-231 sh Ral B                      MDA-MB-231 sh Ral A/B                      MDA-MB-231 sh NE-GFP (Scrambled)                      BT-20 (ATCC HTB-19)                 </div>
<b>Approved Genetic Modifications (Plasmids/Vectors)</b>	Plasmids: pcdna3, pEYFP, pRS, pReceiver-M13	<div style="border: 1px solid blue; padding: 5px; color: blue;">                     PEGFP                      PFLAG-A1                 </div>
<b>Approved Use of Animals</b>	Mouse	
<b>Approved Biological Toxin(s)</b>	Pertussis, Cholera	

Approved Gene  
Therapy

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Approved Plants and  
Insects

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As the Principal Investigator, I have ensured 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>

Signature of Permit Holder:



Current Classification: 2

Containment Level for Added Biohazards: \_\_\_\_\_

Date of Last Biohazardous Agents Registry Form: May 30, 2011

Date of Last Modification (if applicable): \_\_\_\_\_

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

**\*For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.**

Chair, Biohazards Subcommittee:

Date:

**MARCH 2012**

**re: Biosafety info for FI-KISS1R (GPR54) in normal and breast cancer cells**

Recent studies indicate that an increase in KISS1R and GPR54 expression in human breast tumors correlate with more advanced stages of the disease (where it conversely appears to act as a suppressor in many other human cancers)

Stable cell lines were generated using the normal human mammary epithelial cell line MCF10a parent cells and human breast carcinoma cell line MDA-MB-231 parent cells electroporated with FLAG-KISS1R (GPR54) or empty vector control pFLAG A1 (vector has pEGFP-C3 backbone). These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells in media without the antibiotic will expel the construct.) When stimulated with ligand the stable cell lines show significant increase in cell motility (2D/migration assay), increase in the formation of stellate structures (3D/invasion assay), and increased MMP-9 activity as compared to the parent cells.

This information was included in a paper published from this lab: PLoS One 2011;6(6):e21599  
GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness  
Zajac M., et.al.

Proposed by Lab:  
level 1 (in vitro, in vivo)

**MARCH 2012**

**re: Biosafety info for shRNA Ral A & Ral B in breast cancer cells**

Investigation in our lab showed elevated mRNA levels of Ral-GTPases in advanced stages of human breast cancer.

Stable cell lines were generated using the human breast carcinoma cell line MDA-MB-231 parent cells electroporated with shRalA and/or shRalB (constructs commercially available in pRS vector; Origene Technologies). A control non-effective 'scrambled' cell line was also generated. These cell lines require the use of antibiotic (puromycin) for the cells to maintain the transfected shRNA construct. (i.e. Cells exposed to media without the antibiotic will expel the shRNA construct.) The stable cell lines show significant reduction in cell motility (2D/migration assay) as well as a decrease in the formation of stellate structures (3D/invasion assay) as compared to the parent MDA-MB-231 cells.

This information was included in a paper published from this lab: Mol Cancer Res 2009;7(7):1064-77  
{beta}-Arrestin/Ral Signaling Regulates Lysophosphatidic Acid-Mediated Migration and Invasion of Human Breast Tumor Cells  
Timothy T. Li, et.al.

Proposed by lab:  
level 2 (in vitro, in vivo)

**MARCH 2012**

**re: Biosafety info for RalGDS mutant in breast cancer cells**

Investigation in our lab showed elevated mRNA levels of Ral-GTPases in advanced stages of human breast cancer.

Stable cell lines were generated using the human breast carcinoma cell line MDA-MB-231 parent cells electroporated with RalGDS mutant(616-768). A control empty vector cell line was also generated. These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells exposed to media without the antibiotic will expel the construct.) The stable cell lines show significant reduction in cell motility (2D/migration assay) as well as a decrease in the formation of stellate structures (3D/invasion assay) as compared to the parent MDA-MB-231 cells. ie mutant acting as an inhibitor of motility in vitro.

This information was included in a paper published from this lab: Mol Cancer Res 2009;7(7):1064-77  
{beta}-Arrestin/Ral Signaling Regulates Lysophosphatidic Acid-Mediated Migration and Invasion of Human Breast Tumor Cells  
Timothy T. Li, et.al.

Mod Biological Agents March 2012

Add new cell line:

**BT-20** human breast carcinoma ATCC #HTB-19

<http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-19&Template=cellBiology>

Proposed CL by lab:  
level 2 (in vitro, in vivo)



[ATCC Advanced Catalog Search](#) » **Product Details**

### Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's [Material Transfer Agreement](#) (NEW) or, in certain cases, an MTA specified by the depositing institution.

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### Cell Biology

ATCC® Number:

HTB-19™

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**Designations:** BT-20

**Depositors:** EY Lasfargues

**Biosafety Level:** 1

**Shipped:** frozen

**Medium & Serum:** [See Propagation](#)

**Growth Properties:** adherent

**Organism:** *Homo sapiens*

**Morphology:** epithelial

**Source:** **Organ:** mammary gland; breast  
**Disease:** carcinoma

**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:** 1958

**Applications:** BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5. This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices.

**Tumorigenic:** Yes

**Reverse Transcript:** negative

**Antigen Expression:** HLA A1, Bw16 (+/-)

**DNA Profile (STR):** Amelogenin: X  
CSF1PO: 12  
D13S317: 11  
D16S539: 11,14  
D5S818: 12  
D7S820: 10  
THO1: 7,9.3  
TPOX: 11  
vWA: 16,17

**Cytogenetic Analysis:** Normal chromosomes N3, N4, N9, N13, N14, and X may be absent. The markers der(11)t(11;?)(q25;?) (M1); der(1)t(1;3)(p22;p13?) (M2); and der(2)t(2;?) (q37;?) (M5) were detected by W.A. Nelson-Rees, et al., *Int. J. Cancer* 16: 74-85, 1975.

**Isoenzymes:** AK-1, 1-2  
ES-D, 1  
G6PD, B  
GLO-I, 1-2  
PGM1, 1  
PGM3, 1

**Age:** 74 years

**Gender:** female

**Ethnicity:** Caucasian

**Comments:** The cells express the WNT3 and the WNT7B oncogenes [PubMed: 8168088]. This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices. A mycoplasma contaminant was discovered and eliminated early in 1972. Growth of BT-20 cells is inhibited by tumor necrosis factor alpha (TNF alpha). BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5.

**Propagation:** **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.  
**Atmosphere:** air, 95%; carbon dioxide (CO2), 5%  
**Temperature:** 37.0°C

**Subculturing:** **Protocol:**

**Related Links**[NCBI Entrez Search](#)[Make a Deposit](#)[Frequently Asked Questions](#)[Material Transfer Agreement New!](#)[Technical Support](#)[Related Cell Culture Products](#)[Product Information Sheet](#)

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**Cell Line Designation: BT-20**  
**ATCC® Catalog No. HTB-19**

**Table of Contents:**

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

**Cell Line Description**

Organism: *Homo sapiens* (human)  
 Tissue: carcinoma, mammary gland; breast

Age: 74 years  
 Gender: female  
 Ethnicity: Caucasian  
 Growth Properties: adherent  
 Morphology: epithelial  
 DNA profile (STR analysis):  
 Ancestry: X  
 CSF1PO: 12  
 D1S317: 11  
 D16S539: 11,14  
 D5S418: 12  
 D7S820: 10  
 TH01: 7,9,3  
 TPOX: 11  
 vWA: 16,17

Tumorigenic: yes, in nude mice; forms grade II adenocarcinomas  
 Researcher: Tamara L. Sasser, Seattle, WA  
 Available: HTB-19, Bx16 (+/-)  
 Deposited: E.Y. Leifer  
 Passage submitted to the ATCC: 248  
 Comments: This breast tumor line was established by E.Y. Leifer and L. Orzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices. A human carcinoma was discovered and eliminated early in 1972. Growth of BT-20 cells is inhibited by tumor necrosis factor alpha (TNF alpha). BT-20 cells are negative for estrogen receptor but do express an estrogen receptor mRNA that has deletion of 5'. The cells express the WNT5 and the WNT7B oncogenes [PubMed: 8168088].  
 Cytogenetic analysis: Normal chromosomes N3, N4, N9, N13, N14, and X may be absent. The markers del(11)(p12)(q25.2)(M1), der(1)(t(2;22)(p13)(q22), and der(2)(t(2;3)(q37)(q45)) were detected by W.A. Nelson-Rees, et al., *Int. J. Cancer* 16: 74-85, 1975.

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**

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* (Flonig 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](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°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 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 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask. It is important to avoid excessive agitation 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<sub>2</sub> in air atmosphere is recommended if using the medium described on this product.

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.

**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 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°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 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 15 minutes).

Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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 Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

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).

**Cryoprotectant Medium**

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 44-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))
- Sugamman BJ et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. *Science* 230: 943-945, 1985 [PubMed: 86044518]
  - Fogh J et al. Absence of HeLa cell contamination in 169 cell lines derived from human tumors. *J. Natl. Cancer Inst.* 58: 209-214, 1977 [PubMed: 77097006]
  - Lian MS et al. Polypeptide core of a human pancreatic tumor mucin antigen. *Cancer Res.* 50: 2997-3001, 1990 [PubMed: 90242270]
  - Castles CG et al. Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line. *Cancer Res.* 53: 5934-5939, 1993 [PubMed: 94084651]
  - Huguet EL et al. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and

**Cell Line Designation: BT-20**  
**ATCC® Catalog No. HTB-19**

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**Cell Line Description**

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 Tissue: carcinoma, mammary gland; breast

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 Gender: female  
 Ethnicity: Caucasian  
 Growth Properties: adherent  
 Morphology: epithelial  
 DNA profile (STR analysis):  
 Ancestry: X  
 CSF1PO: 12  
 D1S317: 11  
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## Product Information Sheet for HTB-19

disease states of human breast tissue. *Cancer Res.* 54: 2615-2621, 1994 PubMed: 94221588

J. Natl. Cancer Inst. 21: 1131-1147, 1958

Pollack MS et al. HLA-A, B, C and DR allelomorph expression on forty-six cultured human tumor cell lines. *J. Natl. Cancer Inst.* 66: 1003-1012, 1981 PubMed: 81218598

Littlewood-Evans AJ et al. The osteoclast-associated proteinase cathepsin K is expressed in human breast carcinoma. *Cancer Res.* 57: 5386-5390, 1997 PubMed: 98033913

Geiger T et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. *Anti-Cancer Drug Des.* 13: 35-45, 1998

Hsu, R. J., Caputo, J. L., and Masey, 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., Tuhs, J.J. and Vesley, D., (1995) *Laboratory Safety: Principles and Practice*, Second edition, ASM press, Washington, DC.

Centers for Disease Control. (1993). *Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 92-8395*. U.S. Dept. of Health and Human Services; 3rd Edition U.S. Government Printing Office Washington D.C.

### ATCC Warranty

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American Type Culture Collection

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703-365-2700  
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E-mail: [tech@atcc.org](mailto:tech@atcc.org)

**PFLAG A1 VECTOR MAP (BACKBONE PEGFP-C3) MARCH 2012:**

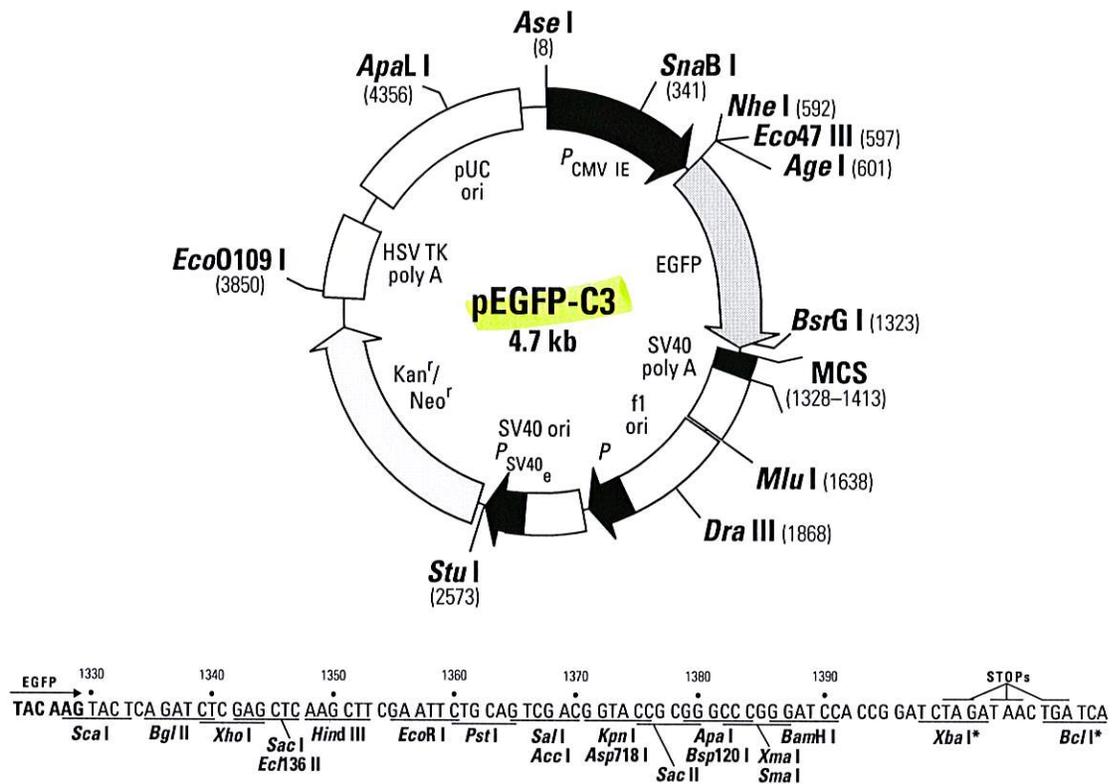
GCTAGCCGGGATGGACTACAAGGACGACCGACGACAAGGGATCCGGAAATTCGGGGTCCGACCTCGAGGGCCGGCTCTAGA **pFLAG-M1**  
GCTAGCCGGGATGGACTACAAGGACGACCGACGACAAGGGATCCGAAATTCGGGGTCCGACCTCGAGGGCCGGCTCTAGA **pFLAG-A1**  
NheI Kozak FLAG BamHI EcoRI Sall XhoI NotI XbaI  
(G/ANNAATGG)

## pEGFP-C3 Vector Information

GenBank Accession #: U57607

PT3052-5

Catalog #6082-1



**Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C3.** All restriction sites shown are unique. The *Bcl* I site cannot be used for fusions since it contains an in-frame stop codon. The *Xba* I and *Bcl* I sites (\*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*<sup>-</sup> host and make fresh DNA.

### Description:

pEGFP-C3 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C3 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C3 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (*Neo*<sup>r</sup>), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-C3 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

**Use:**

Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C3 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C3 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

**Location of Features:**

- Human cytomegalovirus (CMV) immediate early promoter: 1–589  
Enhancer region: 59–465; TATA box: 554–560  
Transcription start point: 583  
C→G mutation to remove *Sac*I site: 569
- Enhanced green fluorescent protein gene  
Kozak consensus translation initiation site: 606–616  
Start codon (ATG): 613–615; Stop codon: 1408–1410  
Insertion of Val at position 2: 616–618  
GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810  
His-231 to Leu mutation (A→T): 1307  
Last amino acid in wild-type GFP: 1327–1329
- MCS: 1328–1413
- SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 1546–1551 & 1575–1580; mRNA 3' ends: 1584 & 1596
- f1 single-strand DNA origin: 1643–2098 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan<sup>r</sup> gene  
–35 region: 2160–2165; –10 region: 2183–2188  
Transcription start point: 2195
- SV40 origin of replication: 2439–2574
- SV40 early promoter  
Enhancer (72-bp tandem repeats): 2272–2343 & 2344–2415  
21-bp repeats: 2419–2439, 2440–2460 & 2462–2482  
Early promoter element: 2495–2501  
Major transcription start points: 2491, 2529, 2535 & 2540
- Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequences:  
Start codon (ATG): 2623–2625; stop codon: 3415–3417  
G→A mutation to remove *Pst*I site: 2805  
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3151
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 3653–3658 & 3666–3671
- pUC plasmid replication origin: 4002–4645

**Primer Locations:**

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

**Propagation in *E. coli*:**

- Suitable host strains: DH5 $\alpha$ , HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number:  $\approx$ 500
- Plasmid incompatibility group: pMB1/ColE1

**References:**

1. Prasher, D. C., *et al.* (1992) *Gene* 111:229–233.
2. Chalfie, M., *et al.* (1994) *Science* 263:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* 341:277–280.
4. Cormack, B., *et al.* (1996) *Gene* 173:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* 6:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125–8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

**Note:** The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

#### Notice to Purchaser

Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

Not-For-Profit research institutes or entities are granted an automatic license with the purchase of this product for use in non-commercial internal research purposes, the terms of which are disclosed in detail in the license that accompanies the shipment of this product. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties.

For-Profit research institutes or entities must obtain a license from Amersham Biosciences. E-mail: [gfp@amershambiosciences.com](mailto:gfp@amershambiosciences.com)

Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support. All companies and institutions purchasing Living Colors™ products will be included in a quarterly report to Aurora Biosciences, as required by the BD Biosciences Clontech/Aurora Biosciences license agreement.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. BD Biosciences Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of BD Biosciences Clontech.

**THE UNIVERSITY OF WESTERN ONTARIO**  
**BIOLOGICAL AGENTS REGISTRY FORM**  
 Approved Biohazards Subcommittee: October 14, 2010  
 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).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) 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/)

PRINCIPAL INVESTIGATOR	<u>Dr. Moshmi Bhattacharya</u>
DEPARTMENT	<u>Physiology &amp; Pharmacology</u>
ADDRESS	<u>1151 Richmond St., Western University N6A 5C1</u>
PHONE NUMBER	<u>X82970</u>
EMERGENCY PHONE NUMBER(S)	<u>519-679-3135</u>
EMAIL	<u>Moshmi.Bhattacharya@schulich.uwo.ca</u>

Location of experimental work to be carried out: Building(s) Medical Science (MSB)  
 Room(s) 224, 231, 235

\*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  
 GRANT TITLE(S): \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

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>
<u>Cynthia Pape</u>	<u>Cynthia.pape@schulich.uwo.ca</u>	<u>11 Oct. 2009</u>
<u>Mistre Alemayehu</u>	<u>malemay@uwo.ca</u>	<u>19 Sept 2009</u>
<u>Jeff Law</u>	<u>jlaw@uwo.ca</u>	<u>26 May 2008</u>
<u>Donna Cvetkovic</u>	<u>dcvetco@uwo.ca</u>	<u>10 Feb 2011</u>
<u>Josh Burley</u>		<u>03 July 2010</u>

Please explain (A) the biological agents and/or biohazardous substances used and (B) how they will be stored, used and disposed of. Projects without this description will not be reviewed.

A) The cell lines will be used to conduct various assays, that are routinely conducted in this laboratory. These include cell migration assays using transwell chambers, immunofluorescence assays to look at the localization of proteins in cells using confocal microscopy, biochemical assays to study protein expression, and motility assays to study the role of specific proteins in cell motility.

B) We have on hand biohazard agents rated Biosafety level 1 and 2, therefore all of our products will be maintained and handled at Biosafety 2 Level requirements. All students/personnel will be properly trained and supervised when handling biohazardous materials. Laboratory space/storage vessels (LN2 tank) containing biohazardous substances will be locked. All cell lines and biohazard substances will be used for research purposes alone and will be handled within a certified biological safety cabinet. All waste products will be disinfected with bleach solution or contained and autoclaved appropriately prior to disposal. Cholera toxin will be inactivated by strong acid solution (2N.HCl) or autoclaved, as recommended by the manufacturer. Pertussis toxin will be autoclaved to inactivate the compound.

Please include a one page research summary or teaching protocol.

My research program focuses on identifying and studying molecules that can be targeted in the treatment of breast cancer metastasis, the leading cause of cancer deaths . Breast cancer is the second leading cause of cancer mortality among Canadian women and it is estimated that 23,200 cases will be diagnosed and 5,300 deaths will occur in Ontario this year as a result of this devastating disease . This translates to 63 new breast cancer diagnoses and 14 breast cancer deaths each day in Ontario. **Identifying appropriate targets for anti-metastatic intervention is therefore essential.**

We have found that G protein-coupled receptors (GPCRs), targets for 60% of all pharmaceuticals, have emerged as crucial players in tumor growth and metastasis . We found that the receptors for bio-active, blood-borne lipid lysophosphatidic acid (LPA) LPA<sub>1</sub> is overexpressed in breast cancer and promote metastatic spread. A key molecule in GPCR function and regulation is the GPCR adaptor protein beta-arrestin. We have recently discovered that beta-arrestins critically regulate LPA<sub>1</sub> receptor mediated breast cancer cell migration and invasion via the small GTPase Ral . Depletion of beta-arrestin or Ral blocks breast cancer invasiveness. **The proposed work will investigate how beta-arrestin regulates cell migration and invasion, and the molecular determinants by which this occurs.** Thus we hypothesize that beta-arrestins promote breast cancer cell invasiveness signaling via small GTPases Ral. The **objectives** of the proposed research are as follows: (I) To establish a role of beta-arrestin signaling in breast cancer metastasis *in vivo* and (II) To determine whether the expression of beta-arrestins and Ral proteins are altered in human tumor samples, and to determine their oncogenic potential.

## 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:

---



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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

---



---

1.2 Please complete the table below:

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
E.coli (DH5alpha cells)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	3L (max 500ml/flask)	Invitrogen	<input checked="" 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

*May 24/11  
Per conversation with C. Page JS*

\*Please attach a Material Safety Data Sheet or equivalent from the supplier.

## 2.0 Cell Culture

2.1 Does your work involve the use of cell cultures?  YES  NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

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		



Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)	LLSG/UH	Not Applicable	none	Not Applicable

#### 4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0?  YES  NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done?  YES, complete table below  NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
<i>DH5alpha (E. coli)</i>	<i>pcdna3</i> <i>pRS</i> <i>pEYFP</i> <i>pReceiver-M13</i>	<i>Invitrogen</i> <i>Origene</i> <i>Clontech</i> <i>GeneCopoeia</i>	<i>Numerous genes will be transfected individually (eg Ral, arrestin, LPA1, LPA1, Rap1); please see papers published from lab (Li et al., Molecular Cancer research 2009; Aziziyeh et al., 2009 Cellular Signalling)</i>	<i>Plasmids commercially available – note websites below</i>

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

\*\* Please attach a plasmid map.

<http://products.invitrogen.com/ivgn/product/V79520?ICID=search-product>

<http://www.origene.com/other/products/TR20003.aspx>

<http://www.origene.com/assets/Documents/msds/HuSHshRNAMaterialSafetyDataSheet.pdf>

[http://www.clontech.com/images/pt/dis\\_vectors/PT3175-5.pdf](http://www.clontech.com/images/pt/dis_vectors/PT3175-5.pdf)

<http://www.genecopoeia.com/tech/omicslink/pReceiver-M13.pdf>

<http://www.ncbi.nlm.nih.gov/pubmed/19609003>

<http://www.ncbi.nlm.nih.gov/pubmed/19306925>

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.

4.4 Will genetic sequences from the following be involved?

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

*May 24/11  
Per conversion  
Sahan  
with  
C. Pepl  
JS.*

4.5 Will virus be replication defective?  YES  NO

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

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

**5.0 Human Gene Therapy Trials**

5.1 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

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

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

5.3 How will the biological agent be administered? \_\_\_\_\_

5.4 Please give the Health Care Facility where the clinical trial will be conducted: \_\_\_\_\_

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used mouse

6.3 AUS protocol # 2008-086-06

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: MDA-MB-231shBarr1; 231 shBarr2; 231shBarr1&2 \_\_\_\_\_  NO

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

Live cells (cell lines) will be injected into the bloodstream of live mice. The mouse body/environment is not optimal for these cells to grow, and it is expected that most of the cells will die. Those cells that do survive will form micro tumours and eventually visible tumours in lung tissue of the mice. Mice will be euthanized and lung tissues collected and fixed for further analysis.

### 7.0 Use of Animal species with Zoonotic Hazards

7.1 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 8.0

7.2 Will live animals be used?  YES  No

7.3 If yes, please specify the animal(s) used:

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

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

---

### 8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)\_\_\_Cholera Toxin (CTX Sigma #C8052)

<http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do>;

Pertussis Toxin (PTX Sigma #P7208)\_

<http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do>\_\_\_\_\_

Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD<sub>50</sub> (specify species) of the toxin\_\_\_CholeraToxin 250ug/kg mouse 260ug/kg i.v.;  
PertussisToxin - rat 114ug/kg i.v. ; mouse 127ug/kg i.v.\_\_\_\_\_

8.4 How much of the toxin is handled at one time\*? \_\_CTX: 50ng ; PTX:  
2ug\_\_\_\_\_

8.5 How much of the toxin is stored\*? \_\_\_\_\_CTX: 1 mg ; PTX: 50ug\_\_\_\_\_

8.6 Will any biological toxins be used in live animals?  YES, Please provide details:\_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)



## 11.0 Import Requirements

11.1 Will any of the above agents be imported?  YES, please give country of origin USA; Sigma-Aldrich takes care of import/export licenses for CTX & PTX; Cedarlane Labs takes care of import/export requirements to obtain cell lines thru ATCC; most recent PHAC acknowledgement of Biosafety level 2 containment 20 May 2010 HPTA #R-06-000598  NO

If no, please proceed to Section 12.0

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

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

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

## 12.0 Training Requirements for Personnel Named on Form

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

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

### 13.0 Containment Levels

13.1 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

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, date of most recent biosafety inspection: 22March2010  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-0122 last inspection 22 March 2010

### 14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.  
Cholera Toxin is inactivated by treatment with mild acid or heat, so will be inactivated by use of 1-2N HCl or by autoclaving. Pertussis toxin will be inactivated thru autoclaving

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

14.2 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:

First aid procedures would involve removal & containment of the product in question from skin/surfaces by washing with cold water to reduce absorption thru pores of the skin or use of eye wash as required: 15-20 minute wash recommended (1 eye wash is located in each of our laboratory spaces). Eye exposure would be followed up with appropriate medical/emergency room visit, as would other exposure if irritation persists. A safety shower is available and recommended for large spills. Animal experiments involving injection of cells into mice would be performed in a certified CL2 containment facility with prior ACVS approval by properly trained personnel on animals that are appropriately restrained for the procedure. Property/waste cloths would be decontaminated with bleach & or autoclaved prior to disposal after the individual affected has been tended to.

\_\_\_\_\_

14.3 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.wph.uwo.ca/>

SIGNATURE  Date: May 24, 2011

### 15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

2) Safety Officer for the University of Western Ontario

SIGNATURE: \_\_\_\_\_

Date: \_\_\_\_\_

*M. Miller*  
21 May 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):

SIGNATURE: \_\_\_\_\_

Date: \_\_\_\_\_

*J Stanley*  
May 07/11

Approval Number: \_\_\_\_\_

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

Special Conditions of Approval:

# Info on Cell Line(s)

## Cell Biology

ATCC® Number:

**HTB-129™**


Designations:

MDA-MB-435S

Biosafety Level:

1

Shipped:

frozen

Medium &amp; Serum:

See Propagation

Growth Properties:

adherent

Organism:

*Homo sapiens* (human)

spindle shaped

Morphology:

**Organ:** previously described as: mammary gland; breast

Source:

**Disease:** previously described as ductal carcinoma**Derived from metastatic site:** pleural effusion

Cellular Products:

tubulin; actin

In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC

Permits/Forms:

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:** 1976

Tumorigenic:

No

Amelogenin: X

CSF1PO: 11

D13S317: 12

D16S539: 13

DNA Profile (STR):

D5S818: 12

D7S820: 8,10

THO1: 6,7

TPOX: 8,11

vWA: 16,18

## Related Links ▶

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----- Original Message -----

**Subject:** Re: Containment Level request: modified MDA-MB-231 cell lines

**Date:** Tue, 22 Sep 2009 11:45:19 -0400

**From:** Jennifer Stanley <jstanle2@uwo.ca>

**To:** genevieve\_lacroix@phac-aspc.gc.ca

Hi Genevieve

Thank you for your voicemail. We will use Level 2 containment for these cells

Jennifer

Jennifer Stanley wrote:

> Hi Genevieve:

>

> I left you a voicemail. We are planning on using these under Level 2  
> containment.

> Regards,

> Jennifer

>

>

>

>

> ----- Original Message -----

> Subject: Re: Containment Level request: modified MDA-MB-231 cell  
> lines

> Date: Wed, 16 Sep 2009 11:37:33 -0400

> From: Geneviève Lacroix <genevieve\_lacroix@phac-aspc.gc.ca>

> To: Jennifer Stanley <jstanle2@uwo.ca>

>

>

>

> Dear Jennifer,

>

> I am sorry for the delayed answer. I believe it would be easier to  
> discuss this case over the phone. Please call me at your convenience.

>

> Regards

>

> Genevieve Lacroix, M.Sc.

> Senior Biosafety Officer / Inspecteur principal, biosécurité Pathogen

> Regulation Directorate (formerly Office of Laboratory Security) /

> Direction de la réglementation des agents pathogènes (anciennement le

> Bureau de sécurité des laboratoires) Public Health Agency of Canada /

> Agence de la santé publique du Canada

> 100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9

> Tel: (613) 946-6982

> Fax: (613) 941-0596

> genevieve\_lacroix@phac-aspc.gc.ca

> <http://www.phac-aspc.gc.ca/ols-bsl/index.html>

>



## Cell Biology

ATCC® Number: **HTB-132™** Order this Item Price: **\$279.00**

Designations: MDA-MB-468  
 Depositors: R Cailleau  
Biosafety Level: 1  
 Shipped: frozen  
 Medium & Serum: [See Propagation](#)  
 Growth Properties: adherent  
 Organism: *Homo sapiens* (human)  
 Morphology: epithelial

Source: **Organ:** mammary gland; breast  
**Disease:** adenocarcinoma

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:** 1977

Applications: transfection host ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Receptors: epidermal growth factor (EGF)  
 transforming growth factor alpha (TGF alpha)

Tumorigenic: Yes

Antigen Expression: Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)

Amelogenin: X

CSF1PO: 12

D13S317: 12

D16S539: 9

DNA Profile (STR): D5S818: 12

D7S820: 8

THO1: 7

TPOX: 8,9

vWA: 18

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modal number = 64; range = 60 to 67.

## Cell Biology

ATCC® Number:	<b>HTB-22™</b>	<a href="#">Order this Item</a>	Price:	<b>\$279.00</b>
Designations:	MCF7		<b>Related Links ▶</b>	
Depositors:	CM McGrath		<a href="#">NCBI Entrez Search</a>	
<a href="#">Biosafety Level:</a>	1		<a href="#">Cell Micrograph</a>	
Shipped:	frozen		<a href="#">Make a Deposit</a>	
Medium & Serum:	<a href="#">See Propagation</a>		<a href="#">Frequently Asked Questions</a>	
Growth Properties:	adherent		<a href="#">Material Transfer Agreement</a>	
Organism:	<i>Homo sapiens</i> (human) epithelial		<a href="#">Technical Support</a>	
Morphology:	 PHOTO		<a href="#">Related Cell Culture Products</a>	
Source:	<b>Organ:</b> mammary gland; breast <b>Disease:</b> adenocarcinoma <b>Derived from metastatic site:</b> pleural effusion <b>Cell Type:</b> epithelial		<b>Login Required ▶</b>	
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5		<a href="#">Product Information Sheet</a>	
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.		<b>BioProducts</b>	
Applications:	transfection host ( <a href="#">Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents</a> )		<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>	
Receptors:	estrogen receptor, expressed			
Antigen Expression:	Blood Type O; Rh+			
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15		<b>BioServices</b>	
			<a href="#">Bio-materials management; basic repository to complex partnership-level services</a>	

modal number = 82; range = 66 to 87.

## Cell Biology

ATCC® Number: **CRL-10317™** [Order this Item](#) Price: **\$279.00**

Designations: MCF 10A

Depositors: Michigan Cancer Foundation

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source: **Organ:** mammary gland; breast

**Disease:** fibrocystic disease

**Cell Type:** epithelial

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:** August 22, 1984

Applications: transfection host ([Roche FuGENE® Transfection Reagents](#))

Tumorigenic: No

Amelogenin: X

CSF1PO: 10,12

D13S317: 8,9

D16S539: 11,12

DNA Profile (STR): D5S818: 10,13

D7S820: 10,11

THO1: 8,9.3

TPOX: 9,11

vWA: 15,17

AK-1, 1 [[23084](#)]

ES-D, 1 [[23084](#)]

G6PD, B [[23084](#)]

Isoenzymes: GLO-I, 1-2 [[23084](#)]

PGM1, 1-2 [[23084](#)]

PGM3, 1 [[23084](#)]

Age: 36 years

Gender: female

Ethnicity: Caucasian

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## Cell Biology

ATCC® Number:	<b>HTB-30™</b>	<a href="#">Order this Item</a>	Price:	<b>\$279.00</b>
Designations:	SK-BR-3		<b>Related Links ▶</b>	
Depositors:	G Trempe, LJ Old		<a href="#">NCBI Entrez Search</a>	
<a href="#">Biosafety Level:</a>	1		<a href="#">Cell Micrograph</a>	
Shipped:	frozen		<a href="#">Make a Deposit</a>	
Medium & Serum:	<a href="#">See Propagation</a>		<a href="#">Frequently Asked Questions</a>	
Growth Properties:	adherent		<a href="#">Material Transfer Agreement</a>	
Organism:	<i>Homo sapiens</i> (human) epithelial		<a href="#">Technical Support</a>	
Morphology:	 PHOTO		<a href="#">Related Cell Culture Products</a>	
Source:	<b>Organ:</b> mammary gland; breast <b>Disease:</b> adenocarcinoma <b>Derived from metastatic site:</b> pleural effusion		<b>Login Required ▶</b>	
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.		<a href="#">Product Information Sheet</a>	
Restrictions:	The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.		<b>BioProducts</b>	
Isolation:	<b>Isolation date:</b> 1970		<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>	
Applications:	transfection host ( <a href="#">Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents</a> )		<b>BioServices</b>	
Tumorigenic:	Yes		<a href="#">Bio-materials management; basic repository to complex partnership-level services</a>	
Antigen Expression:	Blood Type A; Rh+; HLA A11, Bw22(+/-), B40, B18			

Amelogenin: X

## Cell Biology

ATCC® Number: **HTB-36™**  Price: **\$279.00**

Designations: JEG-3

Depositors: G Kohler

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)  
epithelial

Morphology:



Source: **Organ:** placenta  
**Disease:** choriocarcinoma

Cellular Products: human chorionic gonadotropin (hCG), human chorionic somatomammotropin (placental lactogen); progesterone

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Applications: transfection host ([Roche FuGENE® Transfection Reagents](#))

Tumorigenic: Yes

Amelogenin: X,Y

CSF1PO: 11,12

D13S317: 9,11

D16S539: 13,14

DNA Profile (STR): D5S818: 10,11

D7S820: 10,12

THO1: 9,9.3

TPOX: 8

vWA: 16

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Cytogenetic Analysis: This is a hypertriploid human cell line. The modal chromosome

## Cell Biology

ATCC® Number: **HTB-144™** [Order this Item](#) Price: **\$279.00**

Designations: JAR

Depositors: RA Pattillo

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source: **Organ:** placenta

**Disease:** choriocarcinoma

Cellular Products: estrogen; progesterone; human chorionic gonadotropin (hCG); human chorionic somatomammotropin (placental lactogen); hCG production averages 22.5 ng/ml after reculturing

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.

Amelogenin: X,Y

CSF1PO: 7,10

D13S317: 11

D16S539: 9,10

DNA Profile (STR): D5S818: 10,11

D7S820: 10,11

THO1: 6,7

TPOX: 8,11

vWA: 16,18

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This is probably a pseudotriploid human cell line with the modal

## Cell Biology

ATCC® Number:	<b>HTB-133™</b>	<a href="#">Order this Item</a>	Price:	<b>\$279.00</b>
Designations:	T-47D		<b>Related Links ▶</b>	
Depositors:	I Keydar		<a href="#">NCBI Entrez Search</a>	
<a href="#">Biosafety Level:</a>	1		<a href="#">Cell Micrograph</a>	
Shipped:	frozen		<a href="#">Make a Deposit</a>	
Medium & Serum:	<a href="#">See Propagation</a>		<a href="#">Frequently Asked Questions</a>	
Growth Properties:	adherent		<a href="#">Material Transfer Agreement</a>	
Organism:	<i>Homo sapiens</i> (human) epithelial		<a href="#">Technical Support</a>	
Morphology:	 <b>Organ:</b> mammary gland; breast <b>Tissue:</b> duct		<a href="#">Related Cell Culture Products</a>	
Source:	<b>Disease:</b> ductal carcinoma <b>Derived from metastatic site:</b> pleural effusion		<b>Login Required ▶</b>	
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.		<a href="#">Product Information Sheet</a>	
Applications:	transfection host ( <a href="#">Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents</a> ) calcitonin, expressed androgen receptor, expressed estrogen receptor, expressed progesterone receptor, expressed		<b>BioProducts</b>	
Receptors:	glucocorticoid receptor, positive, expressed prolactin, expressed calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin; estrogen receptor, positive		<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>	
			<b>BioServices</b>	
			<a href="#">Bio-materials management; basic repository to complex partnership-level services</a>	
DNA Profile (STR):	Amelogenin: X			

## Cell Biology

ATCC® Number:	<b>CRL-1378™</b>	<a href="#">Order this Item</a>	Price:	<b>\$329.00</b>
Designations:	RBL-1			<b>Related Links ▶</b>
Depositors:	H Metzger, C Isersky			<a href="#">NCBI Entrez Search</a>
<a href="#">Biosafety Level:</a>	1			<a href="#">Make a Deposit</a>
Shipped:	frozen			<a href="#">Frequently Asked Questions</a>
Medium & Serum:	<a href="#">See Propagation</a>			<a href="#">Material Transfer Agreement</a>
Growth Properties:	suspension			<a href="#">Technical Support</a>
Organism:	Rattus norvegicus (rat)			<a href="#">Related Cell Culture Products</a>
Morphology:	lymphoblast			
Source:	<b>Organ:</b> peripheral blood <b>Strain:</b> Wistar <b>Disease:</b> leukemia <b>Cell Type:</b> basophil; chemically induced			<b>BioProducts</b>
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.			<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>
Applications:	transfection host ( <a href="#">Nucleofection technology from Lonza</a> )			
Receptors:	FcERI (Fc of IgE)			<b>BioServices</b>
Comments:	The line exhibits various characteristics of basophil differentiation including surface receptors for IgE. It was shown to not release histamine by an IgE mediated system. PubMed: 6166481			<a href="#">Bio-materials management; basic repository to complex partnership-level services</a>
Propagation:	<b>ATCC complete growth medium:</b> The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. <b>Temperature:</b> 37.0°C			
Subculturing:	<b>Protocol:</b> Cultures can be maintained by the addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 viable cells/ml and maintain between 1 X 10 exp5 cells/ml and 1 X 10 exp6 /ml.			
Preservation:	<b>Medium Renewal:</b> Add medium as cell density increases <b>Freeze medium:</b> Complete growth medium 95%; DMSO, 5% <b>Storage temperature:</b> liquid nitrogen vapor phase			
Related Products:	Recommended medium (without the additional supplements or			

## Cell Biology

ATCC® Number: **CCL-82.1™** [Order this Item](#) Price: **\$329.00**

Designations: GH3

Depositors: AH Tashjian

Biosafety Level: 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: loosely adherent with floating clusters

Organism: Rattus norvegicus (rat)

Morphology: epithelial

Source: **Organ:** pituitary  
**Strain:** Wistar-Furth  
**Disease:** tumor

Cellular Products: prolactin; growth hormone (somatotrophin)

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:** July, 1965

Applications: transfection host ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Virus Susceptibility: Herpes simplex virus  
Vesicular stomatitis virus  
Human poliovirus 1

Cytogenetic Analysis: modal number = 67; range = 47 to 71.  
Stemline karyotype is stable with a few structural alterations. Two dicentric marker chromosomes were observed in 100% of the cells examined.

Age: 7 months

Gender: female

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## Cell Biology

ATCC® Number: **CRL-1651™** [Order this Item](#) Price: **\$279.00**

Designations: COS-7

Depositors: Y Gluzman

Biosafety Level: 2 [Cells Contain SV-40 viral DNA sequences ]

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Cercopithecus aethiops*  
fibroblast

Morphology:  PHOTO

Source: **Organ:** kidney  
**Cell Type:** SV40 transformed

Cellular Products: T antigen

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Applications: transfection host ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Comments: This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region. The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.

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

**Atmosphere:** air, 95%; carbon dioxide (CO<sub>2</sub>), 5%

**Temperature:** 37.0°C

**Protocol:**

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## Cell Biology

ATCC® Number:	<b>CRL-1573™</b>	<a href="#">Order this Item</a>	Price:	<b>\$279.00</b>
Designations:	293 [HEK-293]			<b>Related Links ▶</b>
Depositors:	FL Graham			<a href="#">NCBI Entrez Search</a>
<a href="#">Biosafety Level:</a>	2 [CELLS CONTAIN ADENOVIRUS ]			<a href="#">Cell Micrograph</a>
Shipped:	frozen			<a href="#">Make a Deposit</a>
Medium & Serum:	<a href="#">See Propagation</a>			<a href="#">Frequently Asked Questions</a>
Growth Properties:	adherent			<a href="#">Material Transfer Agreement</a>
Organism:	<i>Homo sapiens</i> (human) epithelial			<a href="#">Technical Support</a>
Morphology:				<a href="#">Related Cell Culture Products</a>
Source:	<b>Organ:</b> embryonic kidney <b>Cell Type:</b> transformed with adenovirus 5 DNA			<b>Login Required ▶</b>
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.			<a href="#">Product Information Sheet</a>
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.			<b><a href="#">BioProducts</a></b>
Applications:	efficacy testing [ <a href="#">92587</a> ] transfection host ( <a href="#">Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents</a> ) virucide testing [ <a href="#">92579</a> ]			<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>
Receptors:	vitronectin, expressed			• <a href="#">sciences</a>
Tumorigenic:	YES			<b><a href="#">BioServices</a></b>
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19			<a href="#">Bio-materials management; basic repository to complex partnership-level services</a>

This is a hypotriploid human cell line. The modal chromosome

## Cell Biology

ATCC® Number:	<b>HTB-26™</b>	<a href="#">Order this Item</a>	Price:	<b>\$279.00</b>
Designations:	MDA-MB-231			<b>Related Links ▶</b>
Depositors:	R Cailleau			<a href="#">NCBI Entrez Search</a>
<a href="#">Biosafety Level:</a>	1			<a href="#">Cell Micrograph</a>
Shipped:	frozen			<a href="#">Make a Deposit</a>
Medium & Serum:	<a href="#">See Propagation</a>			<a href="#">Frequently Asked Questions</a>
Growth Properties:	adherent			<a href="#">Material Transfer Agreement</a>
Organism:	<i>Homo sapiens</i> (human)			<a href="#">Technical Support</a>
	epithelial			<a href="#">Related Cell Culture Products</a>
Morphology:				<b>Login Required ▶</b>
	<b>Organ:</b> mammary gland; breast			<a href="#">Product Information Sheet</a>
	<b>Disease:</b> adenocarcinoma			
Source:	<b>Derived from metastatic site:</b> pleural effusion			
	<b>Cell Type:</b> epithelial			
	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.			
Permits/Forms:				<b>BioProducts</b>
	transfection host ( <a href="#">Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents</a> )			<a href="#">Cell, microbial and molecular genomics products for the life sciences</a>
Receptors:	epidermal growth factor (EGF), expressed			
	transforming growth factor alpha (TGF alpha), expressed			
Tumorigenic:	Yes			
	Amelogenin: X			
	CSF1PO: 12,13			
	D13S317: 13			
	D16S539: 12			<b>BioServices</b>
DNA Profile (STR):	D5S818: 12			<a href="#">Bio-materials management: basic repository to complex partnership-level services</a>
	D7S820: 8,9			
	THO1: 7,9.3			
	TPOX: 8,9			
	vWA: 15,18			

The cell line is aneuploid female (modal number = 64, range = 52 to

# Toxin Info



## TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	0.05 µg
Proposed Storage Dose:	1000 µg
LD <sub>50</sub> (species):	250 µg

<b>Calculation:</b>			
	250 µg/kg	x	50 kg/person
Dose per person based on LD <sub>50</sub> in µg =			12500
LD <sub>50</sub> per person with safety factor of 10 based on LD <sub>50</sub> in µg =			1250

### Comments/Recommendations:



### TOXIN USE RISK ASSESSMENT

<b>Name of Toxin:</b>	Pertussis toxin
<b>Proposed Use Dose:</b>	2 µg
<b>Proposed Storage Dose:</b>	50 µg
<b>LD<sub>50</sub> (species):</b>	114 µg

<b>Calculation:</b>			
	114 µg/kg	x	50 kg/person
Dose per person based on LD <sub>50</sub> in µg =			5700
<b>LD<sub>50</sub> per person with safety factor of 10 based on LD<sub>50</sub> in µg =</b>			<b>570</b>

**Comments/Recommendations:**