

Turley, Eva

Modification Form for Permit BIO-CRCC-006
Permit Holder: Eva Turley

BIO-CRCC-0006

Approved Personnel

(Please stroke out any personnel to be removed)

Jenny Ma

Additional Personnel

(Please list additional personnel here)

Lin Wang
Cory Toelg
Jing Zhang
Colleen Biggs

Please stroke out any approved Biohazards to be removed below

Write additional Biohazards for approval below. *

Approved Microorganisms

E. coli (DH5 alpha), retrovirus moloney murine leukemia virus

HB 101
BL21 (DE3) > E. coli

Approved Cells

rodent (primary), mouse fibroblast cells, human (established), prostatic stem cells, rodent (established), mouse fibroblastic cells

MOA JMO 251, mcf 7,
LOT42, C310T1/2,
Rham-1-MEF, CD44-1-MEF
Rham-1-CD44-1-MEF, Rham + mcf1,
LOT1/2, Rat mesenchymal stem
cells, RAW 264.7, Rat derma
fibroblasts.

Approved Use of Human Source Material

tumour biopsy

Approved GMO

retrovirus; moloney murine leukemia virus, oncogenes RHAM m/HmaR, RAS, mEK1, pBabe vector

Approved use of Animals

mouse lines
rat (until end of Sept. 09)

mouse lines

Approved Toxin(s)

* 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: Jun 18, 2008

Signature of Permit Holder: _____

BioSafety Officer(s): Maile Ryden SEPT 17/09

Chair, Biohazards Subcommittee: _____

Description of Research Use of Cell Lines in Turley Laboratory

E.Coli strains:

HB101
BLz1 (DE3)

These are used for production of recombinant GST-Rhamm protein, which is used for binding analyses of Rhamm to hyaluronan mimetics (to be used for imaging tumor progenitor cells). As well recombinant GST-Rhamm protein is used as an antigen for producing monoclonal and polyclonal anti-Rhamm antibodies. Finally recombinant GST-Rhamm protein is used as a soluble inhibitor of Rhamm function, particularly for in vivo analyses.

Murine (rat and mouse) Cell Lines:

Rat Dermal Fibroblasts
RAW 264.7 (macrophage line)

These murine cell lines are used for in vitro assessment of the consequences of blocking Rhamm antibodies and other reagents on cell motility, proliferation and signaling. Our research using these cell lines focuses upon the consequences of impaired Rhamm function on wound repair. These cell lines were purchased from ATCC and safety sheets have been sent.

10T1/2 fibroblasts
C3-10T1/2 fibroblasts
Rhamm-transfected 10T1/2 fibroblasts

These cell lines are used for tumor studies. We have shown that Rhamm is required for mesenchymal tumor initiation and therefore we use fibroblast cell lines such as 10T1/2 for transfection of Rhamm forms and C3-10T1/2 for transfection of dominant inhibitory forms of Rhamm. These transiently modified fibroblasts are grown in NOD-SCID mice to assess for tumorigenicity. These cell lines are also used for identifying the key signaling pathways controlled by Rhamm that are relevant to tumorigenesis. The 10T1/2 and C3-10T1/2 cell lines were purchased from ATCC and safety sheets have been sent. The Rhamm transfected 10T1/2 fibroblasts were generated in my laboratory.

Rhamm-/- MEF
CD44-/- MEF
Rhamm-/-:CD44-/- MEF

These mouse embryonic fibroblast lines were originally isolated as primary cell cultures and spontaneously immortalized in my laboratory. They are used to identify signaling pathways regulated by Rhamm and CD44 that are relevant to wound repair and tumorigenesis.



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Cell Biology

ATCC® Number:	HTB-26™	<input type="button" value="Order this Item"/>	Price:	\$256.00
Designations:	MDA-MB-231		Related	
Depositors:	R Cailleau		NCBI Entrez	
Biosafety Level:	1		Cell Micrographs	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human)		Technical Support	
Morphology:	epithelial		Related Cell Lines	
				
Source:	Organ: mammary gland; breast Disease: adenocarcinoma Derived from metastatic site: pleural effusion 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.			
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)			
Receptors:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed			
Tumorigenic:	Yes			
DNA Profile (STR):	Amelogenin: X CSF1PO: 12,13 D13S317: 13			

D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, 8
GLO-1, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Gender: female

Ethnicity: Caucasian

Comments: The cells express the WNT7B oncogene [PubMed: 8168088].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Atmosphere: air, 100%

Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C without CO2.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended

Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Related Products: purified DNA:ATCC 45518
purified DNA:ATCC 45519
purified DNA:ATCC HTB-26D
purified RNA:ATCC HTB-26R
recommended serum:ATCC 30-2020
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2008

References: 1206: Brinkley BR, et al. Variations in cell form and cytoskeleton in human

breast carcinoma cells in vitro. *Cancer Res.* 40: 3118-3129, 1980. PubMed: [7000337](#)

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22656: Cailleau R, et al. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In Vitro* 14: 911-915, 1978. PubMed: [730202](#)

22977: Bates SE, et al. Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells. *Endocrinology* 126: 596-607, 1990. PubMed: [2294006](#)

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32489: De Vincenzo R, et al. Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines. *Anticancer Drug Des.* 13: 19-33, 1998. PubMed: [9474240](#)

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The Web site for the CDC's Office of Health and Safety has complete descriptive levels in the text of the publication *Biosafety in Microbiological and Biomedical (BMBL) 5th Edition* (HHS Publication No. (CDC) 93-8395. U.S. Department of Services, Centers for Disease Control and Prevention and National Institutes of Government Printing Office: Washington DC; 2007). It is available in its entire Information on agent risk assessment may be found in the Agent Summary Sheet publication.

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- For further information on the classification of contagious or infectious animal or plant pests, please consult the U.S. Department of Agriculture, Animal Inspection Service.

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Cell Biology

ATCC® Number:	CCL-226™	<input type="button" value="Order this Item"/>	Price:	\$264.00
Designations:	C3H/10T1/2, Clone 8		Related	
Depositors:	C Heidelberger		NCBI Entr	
Biosafety Level:	1		Cell Micro	
Shipped:	frozen		Make a De	
Medium & Serum:	See Propagation		Frequently	
Growth Properties:	adherent		Material I	
Organism:	<i>Mus musculus</i> (mouse)		Technical	
Morphology:	fibroblast		Related Co	
				
Source:	Strain: C3H Organ: embryo			
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:	No			
Reverse Transcript:	negative			
Antigen Expression:	H-2k			
Cytogenetic Analysis:	Mouse karyotype with a modal number of 80 chromosomes.			
Age:	embryo			

Comments: C3H/10T1/2, Clone 8 was isolated by C. Reznikoff, D. Brankow and C. Heidelberger in 1972 from a line of C3H mouse embryo cells. [23019]
The cells are very sensitive to post confluence inhibition of cell division, do not produce tumors in syngeneic mice, have no background of spontaneous transformation, nor do they contain overt endogenous transforming murine leukemia or sarcoma viruses. [22692]
The cells are contact sensitive.
There is no detectable background spontaneous transformation.
They are highly susceptible to transformation by chemical agents. [1208]
Tested and found negative for ectromella virus (mousepox).
NOTE: THE INOCULATION DENSITY, FEEDING AND HARVESTING SCHEDULES MUST BE FOLLOWED RIGIDLY IF THE LINE IS TO RETAIN ITS ESSENTIAL CHARACTERISTICS.
THE BATCH OF SERUM USED FOR GROWTH AND FOR TRANSFORMATION ASSAYS MAY AFFECT BOTH THE MORPHOLOGY OF THIS LINE AND THE RESULTS OBTAINED.
Monolayers established and maintained for the standard transformation assay should be free of all foci after 6 weeks. [1208]
The donor recommends that the line be used between the 5th and 15th passages only.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is Eagle's Basal medium with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate and Earle's BSS. To make the complete growth medium, add the following components to the base medium: heat-inactivated fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing: **Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. SUBCULTURE MUST BE DONE BEFORE THE CULTURE REACHES CONFLUENCE.
Subcultivation Ratio: Seed new flasks at 2000 viable cells/sq cm.
Medium Renewal: Once between subcultures if necessary

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

References: 1208: Reznikoff CA, et al. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. *Cancer Res.* 33: 3239-3249, 1973. PubMed: 4796800
1209: Terzaghi M, Little JB. Repair of potentially lethal radiation damage in mammalian cells is associated with enhancement of malignant transformation. *Nature* 253: 548-549, 1975. PubMed: 1167940
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33039: Jain MK, et al. Molecular cloning and characterization of SmLIM, a developmentally regulated LIM protein preferentially expressed in aortic smooth muscle cells. *J. Biol. Chem.* 271: 10194-10199, 1996. PubMed: 8626582

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Growth Properties:	adherent		Material T	
Organism:	<i>Homo sapiens</i> (human)		Technical	
Morphology:	epithelial		Related C	
				
Source:	Organ: mammary gland; breast Disease: adenocarcinoma Derived from metastatic site: pleural effusion Cell Type: epithelial			
Cellular Products:	Insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5			
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Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)			
Receptors:	estrogen receptor, expressed			
Antigen Expression:	Blood Type O; Rh+			
DNA Profile (STR):	Amelogenin: X			

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CSF1PO: 10
 D13S317: 11
 D16S539: 11,12
 D5S818: 11,12
 D7S820: 8,9
 TH01: 6
 TPOX: 9,12
 vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
 The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per 5 metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

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

Age: 69 years adult

Gender: female

Ethnicity: Caucasian

Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes. The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.

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: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% .
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
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 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended

Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Doubling Time:

29 hrs

Related Products:

purified DNA:ATCC [HTB-22D](#)

purified RNA:ATCC [HTB-22R](#)

0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++) :ATCC [30-2101](#)

Cell culture tested DMSO:ATCC [4-X](#)

recommended serum:ATCC [30-2020](#)

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC [30-2003](#)

References:

21405: Sugarman 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: [3933111](#)

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Drug Des. 13: 35-45, 1998. PubMed: [9474241](#)

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32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. USA 93: 136-140, 1996. PubMed: [8552591](#)

32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: [8650224](#)

38764: Bacus SS, et al. Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. Mol. Carcinog. 3: 350-362, 1990. PubMed: [1980586](#)

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Cell Biology

ATCC® Number:	CRL-1213™	Order this Item	Price:	\$399.00
Designations:	FR		Related	
Depositors:	B Smith		NCBI Entrez	
Biosafety Level:	1		Cell Micrographs	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	Rattus norvegicus (rat)		Technical Support	
Morphology:	fibroblast		Related Cell Lines	
				
Source:	Organ: skin Strain: Sprague-Dawley Disease: normal			
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.			
Age:	18 days gestation			
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%. Temperature: 37.0°C			
Subculturing:	Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for			

culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 10 to 20 minutes).
 Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended

Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium, 95%; DMSO, 5%

Storage temperature: liquid nitrogen vapor phase

Related Products:

Cell culture tested DMSO: ATCC [4-X](#)

recommended serum: ATCC [30-2020](#)

0.25% (w/v) Trypsin - 0.53mM EDTA in Hank's BSS (w/o Ca⁺⁺, Mg⁺⁺):
 ATCC [30-2101](#)

Phosphate-buffered saline: ATCC [30-2200](#)

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC [30-2003](#)

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Cell Biology

ATCC® Number:	CRL-2192™	Order this Item	Price: \$268.00
Designations:	NR8383 [AgC11x3A, NR8383.1]		Related
Depositors:	RJ Helmke		NCBI Entrez
Biosafety Level:	1		Cell Micro
Shipped:	frozen		Make a De
Medium & Serum:	See Propagation		Frequently
Growth Properties:	mixed, adherent and suspension		Material T
Organism:	Rattus norvegicus (rat)		Technical
Morphology:	macrophage		Related_C
	 PHOTO		
Source:	Organ: lung Strain: Sprague-Dawley Disease: normal Cell Type: macrophage (alveolar);		
Cellular Products:	transforming growth factor beta (TGF beta); interleukin 1 (IL-1); interleukin 6 (IL-6)		
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 3, 1983		
Receptors:	Fc		
Comments:	NR8383 (normal rat, August 3, 1983) was established from normal rat alveolar macrophage cells obtained by lung lavage. The cells were cultured in the presence of gerbil lung cell conditioned		

medium for approximately 8 to 9 months.

Subsequently the requirement for exogenous growth factors was lost.

NR8383 cells were cloned and subcloned from single cells by limiting dilution, and then subcloned from soft agar three times.

The cells exhibit characteristics of macrophage cells:

Phagocytosis of zymosan and *Pseudomonas aeruginosa*, nonspecific esterase activity, Fc receptors, oxidative burst, IL-1, TNF beta and IL-6 secretion, and replicative response to exogenous growth factors.

The cells respond to appropriate microbial, particulate or soluble stimuli with phagocytosis and killing.

NR8383 cells respond to bleomycin by secreting latent transforming growth factor (TGF beta).

Stimulation with bleomycin also increases TGF beta mRNA expression.

These cells are sensitive to endotoxin.

LPS levels of 1 to 10 ng/ml inhibit replication by 50%.

LPS inhibition is nontoxic and reversible even after levels up to 0.001 mg/ml for extended periods.

The NR8383 cell line provides a homogenous source of highly responsive alveolar macrophages which can be used in vitro to study macrophage related activities.

Propagation: **ATCC complete growth medium:** Ham's F12K medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 85%; heat inactivated fetal bovine serum, 15%

Temperature: 37.0°C

Subculturing: **Protocol:** Cultures can be maintained by transferring floating cells to additional flasks. Adherent cells may be harvested by scraping. Upon reseeding, about one half of the cells will re-attach. Cultures are most successful when set up at a floating cell concentration of 1 to 4 X 10⁵ exp5 viable cells/ml.

Medium Renewal: Two to three times weekly

Preservation: **Freeze medium:** Complete growth medium, 95%; DMSO, 5%

Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2004
purified RNA: ATCC CRL-2192R

References:
22160: Hidalgo HA, et al. Pneumocystis carinii induces an oxidative burst in alveolar macrophages. *Infect. Immun.* 60: 1-7, 1992. PubMed: 1729174
22316: Helmke RJ, et al. A continuous alveolar macrophage cell line: comparisons with freshly derived alveolar macrophages. *In Vitro Cell. Dev. Biol.* 25: 44-48, 1989. PubMed: 2914814
22674: Helmke RJ, et al. From growth factor dependence to growth factor responsiveness: the genesis of an alveolar macrophage cell line. *In Vitro Cell. Dev. Biol.* 23: 567-574, 1987. PubMed: 3497918
22848: Limper AH, Standing JE. Vitronectin interacts with *Candida albicans* and augments organism attachment to the NR8383 macrophage cell line. *Immunol. Lett.* 42: 139-144, 1994. PubMed: 7534269
22970: Hidalgo HA, et al. The effects of cyclosporine and dexamethasone on an alveolar macrophage cell line (NR8383). *Transplantation* 53: 620-623, 1992. PubMed: 1549855
23173: Denholm EM, Rollins SM. Expression and secretion of transforming growth factor-beta by bleomycin-stimulated rat alveolar macrophages. *Am. J. Physiol.* 264: L36-L42, 1993. PubMed: 7679254
23190: Kriegl DP, et al. Resistance of mucoid *Pseudomonas aeruginosa* to nonopsonic phagocytosis by alveolar macrophages in vitro. *Infect. Immun.* 56: 3173-3169, 1988. PubMed: 3141284
23369: Sherman MP, et al. Pyrrolidine dithiocarbamate inhibits induction of nitric oxide synthase activity in rat alveolar macrophages. *Biochem. Biophys. Res. Commun.* 191: 1301-1308, 1993. PubMed: 7682068
23484: Griscavage JM, et al. Inducible nitric oxide synthase from a rat alveolar macrophage cell line is inhibited by nitric oxide. *J. Immunol.* 151: 6329-6337, 1993. PubMed: 7504017
23566: Henderson SA, et al. Nitric oxide reduces early growth response-1 gene expression in rat lung macrophages treated with interferon-gamma and lipopolysaccharide. *J. Biol. Chem.* 269: 25239-25242, 1994. PubMed: 7523382
36466: Huang S, et al. Rat KC cDNA cloning and mRNA expression in lung macrophages and fibroblasts. *Biochem. Biophys. Res. Commun.* 184: 922-929, 1992. PubMed: 1374243

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Cell Biology

ATCC® Number:	TIB-71™ Order this Item	Price:	\$264.00
Designations:	RAW 264.7	Related Links ▶	
Depositors:	WC Raschke	NCBI Entrez Search	
Biosafety Level:	2	Cell Micrograph	
Shipped:	frozen	Make a Deposit	
Medium & Serum:	See Propagation	Frequently Asked Questions	
Growth Properties:	adherent	Material Transfer Agreement	
Organism:	<i>Mus musculus</i> (mouse)	Technical Support	
Morphology:	monocyte/macrophage	Related Cell Culture Products	
Source:	 <p>Tissue: ascites Strain: BALB/c Disease: Abelson murine leukemia virus-induced tumor Cell Type: macrophage; Abelson murine leukemia virus transformed lysosome [1202]</p>		
Cellular Products:			
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:	Biological response [92560] transfection host (Roche FuGENE® Transfection Reagents)		
Receptors:	complement (C3) [1202]		
Antigen Expression:	H-2d		
Age:	adult		
Gender:	male		
Comments:	This line was established from a tumor induced by Abelson murine leukemia virus. They are negative for surface immunoglobulin (sIg+), Ia (Ia-) and Thy-1.2 (Thy-1.2). This line does not secrete detectable virus particles and is negative in the XC plaque formation assay. The cells will pinocytose neutral red and will phagocytose latex beads and zymosan. They are capable of antibody dependent lysis of sheep erythrocytes and tumor cell targets. LPS or PPD treatment for 2 days stimulates lysis of erythrocytes but not tumor cell targets. Data communicated in Feb. 2007 by Dr Janet W. Hartley, indicates the expression of infectious ecotropic MuLV closely related, if not identical, to the Moloney MuLV helper virus used in the original virus inoculum. The cells also express polytropic MuLV, unsurprisingly based on the mouse passage history of the virus stocks [PubMed 18177500].		

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 (CO₂), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Subcultures are prepared by scraping. For a 75 cm² flask, remove all but 10 ml culture medium (adjust amount accordingly for other culture vessels). Dislodge cells from the flask substrate with a cell scraper; aspirate and add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Replace or add medium every 2 to 3 days.

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

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

References: 1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: [894031](#)
 1207: Raschke WC, et al. Functional macrophage cell lines transformed by Abelson leukemia virus. Cell 15: 261-267, 1978. PubMed: [212198](#)
 32443: Denlinger LC, et al. Regulation of Inducible Nitric oxide synthase expression by macrophage purinoreceptors and calcium. J. Biol. Chem. 271: 337-342, 1996. PubMed: [8550583](#)
 32466: Hambleton J, et al. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad. Sci. USA 93: 2774-2778, 1996. PubMed: [8610116](#)
 32553: Taylor GA, et al. Identification of a novel GTPase, the inducibly expressed GTPase, that accumulates in response to interferon gamma. J. Biol. Chem. 271: 20399-20405, 1996. PubMed: [8702776](#)
 32901: Li YM, et al. Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc. Natl. Acad. Sci. USA 93: 11047-11052, 1996. PubMed: [8855306](#)
 33046: Panneerselvam K, Freeze HH. Mannose enters mammalian cells using a specific transporter that is insensitive to glucose. J. Biol. Chem. 271: 9417-9421, 1996. PubMed: [8621609](#)
 33076: Lokuta MA, et al. Mechanisms of murine RANTES chemokine gene induction by Newcastle disease virus. J. Biol. Chem. 271: 13731-13738, 1996. PubMed: [8662852](#)
 33162: Taylor MF, et al. In vitro efficacy of morpholino-modified antisense oligomers directed against tumor necrosis factor-alpha mRNA. J. Biol. Chem. 271: 17445-17452, 1996. PubMed: [8663413](#)
 92560: Standard Practice for Testing for Biological Responses to Particles in Vitro. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 1903-98R03.

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CANCER RESEARCH/LRCC
Attn: Jennifer

BIO-LRCC-0006 @001

Fax 519-661-3420

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr. Eva Turley
SIGNATURE [Signature]
DEPARTMENT CRL LRCC
ADDRESS 800 Commissioner Rd. East.
PHONE NUMBER 519-686-8500
EMAIL Eva.Turley@LHSC.on.ca

Location of experimental work to be carried out: Building(s) A4-824 (etc) Room(s) A4-931
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Roberts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

- TITLE OF GRANT(S):
1. Use of HA-metal nanoparticles to identify and characterize tumor progenitor cell subsets in breast tumors.
2. The role of RHAMM in Wound Repair.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES Dept. of Defense / CDMRP / COBER / LRCC / C.I.N.O. / LHRI

- Names of all personnel working under Principal Investigators' supervision in this location:
i) Jenny Ma
ii) Jing Zhang
iii) Yuan Qi
iv) Colleen Biggs
v) _____

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? YES NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
<i>Salmonella</i> E. Coli	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	0.5 L @ 0.70 D.
Retrovirus	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No <i>only cats</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	10 ml
Monkey Nucleo	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Monkey Nucleo Virus	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required.

1(2) 3

1.4 Source of microorganism(s) or biological agent(s)? In vitro

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures?
If no, please proceed to Section 3.0

YES NO

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Mouse Fibroblast Cells
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	
Other (specify)		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Human Prostat. Stem Cells	Lab in US (McGill)
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Mouse Fibroblastic Cells	Our Mice
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No	Phoenix (packaging cell line)	Portland Lab
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1(2) 3

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____
 YES NO

5.5 Has human ethics approval been obtained? YES NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? YES NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 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 _____
colony # _____

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 _____

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

9.0 Import Requirements

9.1 Will the agent be imported? YES NO

If no, please proceed to Section 10.0
If yes, country of origin _____

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

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

9.4 Has the Import permit been sent to OHS?
If yes, Permit # _____ YES NO

10.0 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

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 8.0 have been trained.

SIGNATURE _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 (2) 3

11.2 Has the facility been certified by OHS for this level of containment? YES NO

11.3 If yes, please give the date and permit number: April 24, 2008 (30)

12.0 Approvals

UWO Biohazard Subcommittee SM. Kelder 19 June '08

Signature [Signature] Date Jan 18, 2008

Safety Officer for Institution where experiments will take place

Signature [Signature] Date April 24, 2008

Safety Officer for University of Western Ontario (if different than above)

Signature [Signature] Date June 18/08