THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	DR. F. BEIER
SIGNATURE	
DEPARTMENT	PHYSIOLOGY AND PHARMACOLOGY.
ADDRESS	DENTAL SCIENCES BLDG RM 00
PHONE NUMBER	x 85344
EMERGENCY PHONE NUMBER(S)	(519) 474-1552
EMAIL	fbeier@uwo.ca.

Location of experimental work to be carried out: Building(s) DSB Room(s) 0033, C029

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Raf function in endochondral ossification (2008-2013)
Transformive growth factor alpha in a rodent model of osteoarthritis (2008-2011)
Regulation of endochondral bone development by glucocorticoid signaling (2007-2010)

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED. APPENDED ON FOLLOWING PAGE.

Names of all personnel working under Principal Investigators supervision in this location:

VERONICA ULICI
GUOYAN WANG
RYAN GILLESPIE
SHIRINE USMANI
LAUREN SOLOMON
RYAN PERLUS
EMILY LE Blanc
SADIA LADHANI
CHANTAL TACCHINO
JENNIFER LI
QIAN YAN
DAWN BRYCE
HOLLY DUPUIS

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Grant funding (all from CIHR)

- Rac1 function in endochondral ossification (2008-2013)
- Transforming growth factor alpha in a rodent model of osteoarthritis (2008-2011)
- Regulation of endochondral bone development by glucocorticoid signaling (2007-2010)

Summary of research:

We are interested in the processes controlling skeletal development, homeostasis and pathology (e.g. osteoarthritis). To study these processes, we are using wild type and genetically modified mice, as well as cells and tissues isolated from these mice. Genetically modified mice include KO lines for Rac2, Akt1, eNos, iNos, TGFalpha and Roralpha genes, conditional mutants for Rac1, ATRX, GSK3B and the glucocorticoid receptor, and transgenic mice expressing Cre recombinase in cartilage, bone or limb. Primary cells from the mice in cell culture will be infected with adenoviral vectors to modify gene expression. In parallel, we are using selected mammalian cell lines to study the physiology of skeletal cells.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES ⊘ NO
(including but not limited to 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? O YES O NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____
Please attach the CFIA permit.
Please describe any CFIA permit conditions:
_____
_____
_____

1.2 Please complete the table below:

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? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*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 O 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	⊘ Yes O No	Dr. D. Hess / Roberts.	Not applicable
Rodent	⊘ Yes O No	Mouse chondrocytes - UWO Animal Facility/Charles River	2006-024 2007-045
Non-human primate	O Yes ⊘ No		
Other (specify)	O Yes ⊘ No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	✓ Yes O No	Hek293	ATCC LEVEL 2
Rodent	✓ Yes O No	ATDC5 NIH3T3, MC3T3, RAW264	RIKEN CELL BANK ATCC
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ✓ YES O NO
If no, please proceed to Section 4.0

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	BONE MARROW MESENCHYMAL CELLS DR. D. HESS/ROBARTS	O Yes ✓ No		O 1 ✓ 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

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 O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

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

4.3 Will genetic modification(s) involving viral vectors be made? ⊘ YES, complete table below O NO

Virus Used for Vector (backbone) Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Adenovirus-Types (dE1/E3)	Ad-Cre-GFP Ad-GFP	VectorBiolabs	eGFP (fluorescent marker) Cre recombinase	excision of sequences within flanking loxP sites

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ⊘ YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O 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 Will any of the agents listed be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊘NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 ⊘ NO

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

SIGNATURE _____

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus BIO-UWO-C085
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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 25, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045
http://www.vectorbiolabs.com

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

3701 Market Street, Suite 340, Philadelphia, PA 19104 Tel: 877-biolabs Fax: 215-966-6001 info@vectorbiolabs.com

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Product Description

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Cell Biology

ATCC® Number: CRL-1658™ Order this Item
Price: $256.00

Designations: NIH/3T3

Biosafety Level: 1

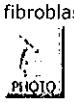
Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: fibroblast



Source:
Organ: embryo
Strain: NIH/Swiss
Cell Type: fibroblast fibroblast;

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)

Virus Susceptibility: Murine leukemia virus

Age: embryo

Comments: The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457].
Tested and found negative for ectromelia virus (mousepox).

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: bovine calf serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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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 which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less.
Subcultivation Ratio: Inoculate 3 to 5 X 10(3) cells/cm2
Medium Renewal: Twice per week

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

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

References:

22370: Jainchill JL, et al. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. 4: 549-553, 1969. PubMed: 4311790

26133: Andersson P, et al. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. Cell 16: 63-75, 1979. PubMed: 84715

26134: Copeland NG, Cooper GM. Transfection by exogenous and endogenous murine retrovirus DNAs. Cell 16: 347-356, 1979. PubMed: 222457

28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321

32372: Berson JF, et al. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus tyep 1 strains. J. Virol. 70: 6288-6295, 1996. PubMed: 8709256

32478: Jones PL, et al. Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. Mol. Cell. Biol. 17: 6970-6981, 1997. PubMed: 9372929

32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. J. Virol. 70: 7921-7928, 1996. PubMed: 8892915

32522: Siess DC, et al. Exceptional fusogenicity of chinese hamster ovary cells with murine retrovirus suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process. J. Virol. 70: 3432-439, 1996. PubMed: 8648675

32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. J. Biol. Chem. 271: 24105-24114, 1996. PubMed: 8798649

32557: Medin JA, et al. Correction in trans for Fabry disease: expression, secretion, and uptake of alpha-galactosidase A in patient-derived cells driven by a high-titer recombinant retroviral vector. Proc. Natl. Acad. Sci. USA 93: 7917-7922, 1996. PubMed: 8755577

32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812

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

32702: Cranmer LD, et al. Identification, analysis, and evolutionary relationships of the putative murine cytomegalovirus homologs of the human cytomegalovirus UL82 (pp71) and UL83 (pp65) matrix phosphoproteins. J. Virol. 70: 7929-7939, 1996. PubMed: 8892916

32724: Shisler J, et al. Induction of susceptibility to tumor necrosis factor by E1A is dependent on binding to either p300 or p105-Rb and induction of DNA synthesis. J. Virol. 70: 68-77, 1996. PubMed: 8523594

32756: Cavanaugh VJ, et al. Murine cytomegalovirus with a deletion of genes spanning HindIII-J and -I displays altered cell and tissue tropism. J. Virol. 70: 1365-1374, 1996. PubMed: 8627652

32905: Westerman KA, Leboulch P. Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination. Proc. Natl. Acad. Sci. USA 93: 8971-8976, 1996. PubMed: 8799138

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CELL SEARCH SYSTEM

RCB0565 : ATDC5	
Comment	Differentiate to chondrocytes, unidentifiable pigment cells. Teratocarcinoma AT805 derived.
Animal	mouse, 129
Sex	Male
Age	embryo
Tissue	embryo
Morphology	epithelial-like
Anchoraged	Yes
Medium	(DMEM:HamF12=1:1)+5%FBS
Antibiotics	Free
Growth temp	37°C
CO_2 concentration	5%
Passage method	0.25% trypsin
Split ratio	1:8 split
Subculture frequency	SC or MC : once/2-3days
Cloned	Yes
Lifespan	infinite
Mycoplasma	-
Isozyme analysis	LD, NP
Originator	Atsumi, Tadao
Registor	Atsumi, Tadao
Restriction	No restriction regarding academic use. a
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User's Publication	2272 2487 2488 2874 2899 2900 2926 3120 3158 3194 3330 3584

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Cell Biology

ATCC® Number: CRL-1573™ Order this Item
Price: $256.00

Designations: 293 [HEK-293]
Depositors: FL Graham
Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Homo sapiens* (human)
Morphology: epithelial

PHOTO

Source: **Organ:** embryonic kidney
Cell Type: transformed with adenovirus 5 DNA
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.
Restrictions: These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications: efficacy testing [92587]
transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
viruscide testing [92579]
Receptors: vitronectin, expressed
Tumorigenic: Yes
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
Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der (1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age: fetus

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Comments:
Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses.
The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

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
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

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.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: 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:
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D
Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006

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ATCC® Number:	**CRL-2593™** Order this Item	**Price:**	**$318.00**
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Morphology:	fibroblast		
Source:	**Organ:** bone **Strain:** C57BL/6 **Tissue:** calvaria **Cell Type:** preosteoblast;		
Cellular Products:	collagen [51540]		
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Tumorigenic:	Yes		
Age:	newborn newborn		
Comments:	A series of subclones were isolated from the cloned but phenotypically heterogeneous MC3T3-E1 cell line. The subclones were selected for high or low osteoblast differentiation and mineralization after growth in medium containing ascorbic acid. The MC3T3-E1 Subclone 4 (ATCC CRL-2593) and the MC3T3 Subclone 14 (ATCC CRL-2594) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate. They form a well mineralized extracellular matrix (ECM) after 10 days [PubMed ID: 10352097]. The MC3T3 Subclone 24 (ATCC CRL-2595) and the MC3T3 Subclone 30 (ATCC CRL-2596) lines exhibit poor osteoblast differentiation after growth in ascorbic acid. They do not form ECM. They can be used as negative controls for Subclones 4 and 14 [PubMed ID: 10352097]. Mineralizing subclones selectively express mRNAs for the osteoblast markers, bone sialoprotein (BSP), osteocalcin (OCN), and the parathyroid hormone (PTH)/parathyroid hormone-related protein (PTHrP) receptor.S ubclones with both high and low differentiation potential produce similar amounts of collagen in culture and express comparable basal levels of mRNA encoding Osf2/Cbfa1, an osteoblast-related transcription factor [PubMed ID: 10352097]. After implantation into immunodeficient mice, highly differentiating subclones form bone-like ossicles resembling woven bone, while poorly differentiating cells only produce fibrous tissue [PubMed ID: 10352097]. These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behavior similar to primary calvarial osteoblasts.		

Propagation:	**ATCC complete growth medium:** The base medium for this cell line is Alpha Minimum Essential Medium with ribonucleosides, deoxyribonucleosides, 2 mM L-glutamine and 1 mM sodium pyruvate, but without ascorbic acid (GIBCO, Custom Product, Catalog No. A1049001). 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:** 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 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 37C. **Subcultivation Ratio:** A subcultivation ratio of 1:6 to 1:8 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitogen vapor phase
Related Products:	recommended serum:ATCC 30-2020 derived from same cell line:ATCC CRL-2595 derived from same cell line:ATCC CRL-2594 derived from same cell line:ATCC CRL-2596 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
References:	51540: Wang D, et al. Isolation and characterization of MC3T3-E1 preosteoblast subclones with distinct in vitro and in vivo differentiation/mineralization potential. J. Bone Miner. Res. 14: 893-903, 1999. PubMed: 10352097

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Designations: RAW 264.7
Depositors: WC Raschke
Biosafety Level: 2
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)
Morphology: monocyte/macrophage

Source:
Tissue: ascites
Strain: BALB/c
Disease: Abelson murine leukemia virus-induced tumor
Cell Type: macrophage; Abelson murine leukemia virus transformed

Cellular Products: lysozyme [1207]

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) [1207]

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].

Price: $264.00

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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 anovel GTPase, the inducibly expresed 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 newcatle disease virus. J. Biol. Chem. 271: 13731-13738, 1996. PubMed: 8662857 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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