Modification Form for Permit BIO-UWO-0148

Permit Holder: John DiGuglielmo

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

Eddie Chan
Boun Thai
Ciric To
Sarah McLean
Adrian Gunaratne

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. Give the full name - do not appreviate.**
Approved Microorganisms	E.Coli DH5 alpha	
Approved Primary and Established Cells	Human (established) - HEK 293, HEK 293T, HeLa, IlepG2 Rodent (established) - Rat 2, Mv1Lu, ,C2C12, NIH/3T3. Non-human primate (established) - Cos7, Cos 1. Others (established) - HDCK. NTCC line H1299.	A549 - NON SMALL CELL LUNG CANCER CELLS FROM ATCC BIOSAFETY LEVEL 1 CELLS
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] - pCMV5, pIRES2-GFP, paxillin-EGFP.	
Approved Use of Animals		
Approved Biological 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 STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: JOHN DI GIGLIANO

Current Classification: 2 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Oct 27, 2009

Date of Last Modification (if applicable): May 14, 2010

BioSafety Officer(s):

Chair, Biohazards Subcommittee: **Date:**

These cells will be cultured in a dedicated culture lab. We normally use level 2 precautions with all our cell line even though the 1549 cells are level 1.
We plan to transfect these cells with various constructs and assess their ability to proliferate, migrate and signal via the TGFβ receptor. Normally, cells will be lysed using standard lysis buffers and immunoblotted with various antibodies.

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Product Description

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

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Cell Biology

ATCC® Number: CCL-185™ (Order this Item) **Price:** $256.00

Designations: A549

Depositors: M Lieber

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source: **Organ:** lung
Disease: carcinoma

Cellular Products: keratin

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:** 1972

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

DNA Profile (STR): Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
THO1: 8,9.3
TPOX: 8,11
vWA: 14

Cytogenetic Analysis: This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6)(q11;q27); ?del(6) (p23); del(11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.

Related Links

NCBI Entrez Search

Cell Micrograph

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Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Isoenzymes:	G6PD, B
Age:	58 years
Gender:	male
Ethnicity:	Caucasian
Comments:	This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218] Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030] The cells are positive for keratin by immunoperoxidase staining.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 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 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. Cultures can be established between 2 X 10(3) and 1 X 10(4) viable cells/cm2. Do not exceed 7 X 10(4) cels/cm2. 6. Incubate cultures at 37°C. **Interval:** Maintain cultures at a cell concentration between 6 X 10(3) and 6 X 10(4) cell/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 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:	about 22 hours
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020

References:

23218: Giard DJ, et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. 51: 1417-1423, 1973. PubMed: 4357758
27669: Mayr GA, Freimuth P. A single locus on human chromosome 21 directs the expression of a receptor for adenovirus type 2 in mouse A9 cells. J. Virol. 71: 412-418, 1997. PubMed: 8985365
27819: Goodrum FD, Ornelles DA. The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle. J. Virol. 71: 548-561, 1997. PubMed: 8985383
32299: St. Geme JW, et al. Characterization of the genetic locus encoding Haemophilus influenzae type b surface fibrils. J. Bacteriol. 178: 6281-6287, 1996. PubMed: 8892830
32347: Horikami SM, et al. The Sendai virus V protein interacts with the NP protein to regulate viral genome RNA replication. Virology 222: 383-390, 1996. PubMed: 8806522
32351: Huang S, et al. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. J. Virol. 70: 4502-4508, 1996. PubMed: 8676475
32373: Goodrum FD, et al. Adenovirus early region 4 34-kilodalton protein directs the nuclear localization of the early region 1B 55-kilodalton protein in primate cells. J. Virol. 70: 6323-6335, 1996. PubMed: 8709260
32394: Fang R, Aust AE. Induction of ferritin synthesis in human lung epithelial cells treated with crocidolite asbestos. Arch. Biochem. Biophys. 340: 369-375, 1997. PubMed: 9143343
32488: 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. Anticancer Drug Des. 13: 35-45, 1998. PubMed: 9474241
32496: Evdokiou A, Cowled PA. Tumor-suppressive activity of the growth arrest-specific gene GAS1 in human tumor cell lines. Int. J. Cancer 75: 568-577, 1998. PubMed: 9466658
32511: Giavedoni LD, Yilma T. Construction and characterization of replication-competent simian immunodeficiency virus vectors that express gamma interferon. J. Virol. 70: 2247-2251, 1996. PubMed: 8642649
32514: Bartz SR, et al. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. 70: 2324-2331, 1996. PubMed: 8642659
32722: Garofalo R, et al. Transcriptional activation of the interleukin-8 gene by respiratory syncytial virus infection in alveolar epithelial cells: nuclear translocation of the RelA transcription factor as a mechanism producing airway mucosal inflammation. J. Virol. 70: 8773-8781, 1996. PubMed: 8971006
32758: Jamaluddin M, et al. Inducible translational regulation of the NF-IL6 transcription factor by respiratory syncytial virus infection in pulmonary epithelial cells. J. Virol. 70: 1554-1563, 1996. PubMed: 8627674
33091: Lewis JA, et al. Inhibition of mitochondrial function by interferon. J. Biol. Chem. 271: 13184-13190, 1996. PubMed: 8662694
58030: Lieber M, et al. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer 17: 62-70, 1976. PubMed: 175022

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Modification Form for Permit BIO-UWO-0148

Permit Holder: John DiGuglielmo

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

Eddie Chan

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E.Coli DH5 alpha	
Approved Cells	Human (established) - HEK 293, HEK 293T, HeLa, IlepG2 Rodent (established) - Rat 2, Mv1Lu, ,C2C12, NIH/3T3. Non-human primate (established) - Cos7, Cos 1. Others (established) - HDCK.	ATCC line. H1299
Approved Use of Human Source Material		
Approved GMO	[Plasmid] - pCMV5, pIRES2-GFP	[PLASMID] - Paxillin -EGFP
Approved use of Animals		
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.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Oct 27, 2009

Date of Last Modification (if applicable): _____

BioSafety Officer(s): JStanley May 14, 2010

Chair, Biohazards Subcommittee: E.M. Kidder

BRIEF DESCRIPTION OF WORK TO BE DONE W/ Paxillin-GFP:

GFP - Rat2 fibroblast cells will be transfected with the paxillin-GFP vector using calcium phosphate or Lipofectamine. Following incubation at 37°C for 24-36 hrs, cells will be analyzed using a fluorescent microscope equipped with temperature and CO_2 controllers. The movement of paxillin-GFP will be assessed in living cells.

H1299 - Briefly, H1299 lung carcinoma cells will be used to assess cell migration and invasion in in vitro cell culture assays. Various drugs that target the cell cytoskeleton will be used to block migration.

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Product Description

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: CRL-5803™ Order this Item
Price: $269.00

Designations: NCI-H1299
Depositors: AF Gazdar, JD Minna
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Homo sapiens* (human)
Morphology: epithelial
Source: **Organ:** lung
Disease: carcinoma; non-small cell lung cancer
Derived from metastatic site: lymph node
Cellular Products: neuromedin B
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: The line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.
Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
DNA Profile (STR): Amelogenin:X
CSF1PO:12
D13S317:12
D16S539:12,13
D5S818:11
D7S820:10
THO1:6,9.3
TPOX:8
vWA: 16,17,18
Age: 43 years adult
Gender: male
Ethnicity: Caucasian
Comments: The cells have a homozygous partial deletion of the p53 protein, and lack expression of p53 protein. They reported to be able to synthesize the peptide neuromedin B (NMB) at 0.1 pmol/mg protein, but not the gastrin releasing peptide (GRP).

Related Links
NCBI Entrez Search
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Material Transfer Agreement
Technical Support
Related Cell Culture Products

Plasmid 15233: Paxillin-pEGFP

Gene/insert name:	paxillin
Insert size (bp):	1700
GenBank/Entrez ID of insert:	L30099
Gene/insert aliases:	PXN
Species of gene(s):	G. gallus (chicken)
Fusion proteins or tags:	GFP
Terminal:	C terminal on backbone
Vector backbone:	pEGFP-N3 (Search Vector Database)
Backbone manufacturer:	Clontech
Type of vector:	Mammalian expression
Backbone size (bp):	4700
Cloning site 5':	Bgl II
Site destroyed during cloning:	Yes
Cloning site 3':	Kpn
Site destroyed during cloning:	No
5' Sequencing primer:	CMV immediate early gene forward primer (List of Sequencing Primers)
Bacteria resistance:	Kanamycin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Rick Horwitz
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge

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	JOHN DiGUGLIELMO
SIGNATURE	
DEPARTMENT	PHYSIOLOGY & PHARMACOLOGY
ADDRESS	MEB, SCI. BLDG. RM 225
PHONE NUMBER	80242
EMERGENCY PHONE NUMBER(S)	519 642-2858
EMAIL	JOHN.DIGUGLIELMO@SCHULICH.UWO.CA

Location of experimental work to be carried out: Building(s) MSB Room(s) 222, 225

*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 / NSERC
GRANT TITLE(S):
1) CIHR: TGFβ RECEPTOR TRAFFICKING AND SIGNALING IN LUNG CANCER CELLS
2) NSERC: THE MECHANISM OF TGFβ RECEPTOR DEGRADATION VIA LIPID RAFTS

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.

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

1) BOON THAI
2) CIRIC TO
3) ADRIAN GUMARANG
4) SARAH MCLEAN
5) EDDIE CHAN

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓YES O 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 ✓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
E. coli DH5α	O Yes ✓No	O Yes ✓No	O Yes ✓No	500 ml.		✓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	ESTABLISHED CELL LINES	Not applicable
Rodent	✓Yes O No	" " "	
Non-human primate	✓Yes O No	" " "	
Other (specify)	✓Yes O 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	HEK 293, HEK 293T, HeLa, HepG2	ATCC
Rodent	☑ Yes O No	Rat2, MDTF, P2C12, NIH3T3	"
Non-human primate	☑ Yes O No	Cos 7, Cos 1	"
Other (specify)	☑ Yes O No	MDCK	"

*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 O 1 ☑ 2 O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material 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		O Yes O No		O 1 O 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 O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α	pCMV5 pIRES2 - GFP	S. WRIGHT, UOFT CLONTECH	PARG	CELLS WILL BECOME MORE MOTILE IN CELL CULTURE

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* 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? O YES O NO N/A

4.6 Will virus be infectious to humans or animals? O YES O NO N/A

4.7 Will this be expected to increase the containment level required? O YES O NO N/A

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 ⊘NO

5.3 How will the biological agent be administered? N/A

5.4 Please give the Health Care Facility where the clinical trial will be conducted: N/A

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING N/A

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 N/A

* 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 ☑ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus BIO-UWO-0148
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: Sept 30'09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 27 Oct 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Oct 26/09

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

Approval Number: BIO-UWO-0148 Expiry Date (3 years from Approval): October 26, 2012

Special Conditions of Approval:

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

The work to be carried out in the lab will require making point mutations in the Par6 gene and introducing the new constructs into mammalian cells.

Briefly, we will amplify Par6 cDNA (in am ampicillin-resistant pCMV5 vector) in DH5α E coli. The pCMV5-Par6 constructs will be purified using a Qiagen maxi-prep column and will be used as a template for site directed mutagenesis. This will be carried out in a one-step PCR protocol using a kit from Invitrogen, using primers containing 2-3 base pair substitutions. We will generate mutations in the Par6 construct whereby lysine at position 19 will be replaced with an alanine. This K19 mutant (also in pCMV5 vector) will also be amplified in DH5α and purified using a Qiagen maxi-prep kit.

The wild-type and K19 Par6 cDNA will then be excised using restriction enzymes and ligated in an pIRES-GFP construct that will express Par6 (wt and K19) and GFP in mammalian cells. Following calcium phosphate-mediated transfection into Rat2 fibroblasts, cells will be challenged with G418 which will select for cells containing pIRES-GFP (control), pIRES-Par6-WT or pIRES-Par6-K19. The stable cell lines will then be ring cloned and we will carry out scratch assays to assess if the mutated Par6 K19 construct alters cell migration.

invitrogen

Material Safety Data Sheet

Revision Date: 09-Nov-2006

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	5-10

The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.

Form
Liquid

Principle Routes of Exposure/
Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures — Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Glycerol	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

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Cell Biology

ATCC® Number:	CRL-1573™ (Order this Item)
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
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

Price: $256.00

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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(17)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

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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Cell Biology

ATCC® Number:	CRL-11268™ (Order this Item)
Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	Organ: kidney
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:	The line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
Age:	fetus

Price: $264.00

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Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.
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%. **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. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:8 is recommended **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:	recommended serum:ATCC 30-2020 derivative:ATCC CRL-11269 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
References:	45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361 57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999 57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001 57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

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Cell Biology

ATCC® Number:	CCL-2™ Order this item
Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 (CELLS CONTAIN PAPOVAVIRUS)
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (Lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
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 ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 TH01: 7 TPOX: 8,12 vWA: 16,18

Price: $256.00

Related Links
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Cytogenetic Analysis:	Modal number = 82, range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of M1, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
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:** 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. **Subcultivation Ratio:** A subcultivation ratio of 1:2 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
Related Products:	recommended serum:ATCC 30-2020 derivative:ATCC CCL-2.2 derivative:ATCC CCL-2.3 derivative:ATCC CCL-2.1 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
Bioreactive Factors:	**Growth Factors:** T cell growth factor (TCGF)

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Cell Biology

ATCC® Number:	HB-8065™ (Order this Item)	Price:	$264.00
Designations:	Hep G2		Related Links ▶
Depositors:	Wistar Institute		NCBI Entrez Search
Biosafety Level:	1		Cell Micrograph
Shipped:	frozen		Make a Deposit
Medium & Serum:	See Propagation		Frequently Asked Questions
Growth Properties:	adherent		Material Transfer Agreement
Organism:	*Homo sapiens* (human)		Technical Support
Morphology:	epithelial		Related Cell Culture Products
	photo		
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma		
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; [3525] complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein) [3525]		
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:	insulin; insulin-like growth factor II (IGF II) [22446]		
Tumorigenic:	No		

DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 F13A01: 5,7 F13B: 6,10 FESFPS: 11 LPL: 10,11 THO1: 9 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	modal number = 55 (range = 50 to 60); has a rearranged chromosome 1 [3525]
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. [23557] The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to granoxere (oxidative stress). [26594] There is no evidence of a Hepatitis B virus genome in this cell line. [1205] [22909]
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:** 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. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 is recommended **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 serum:ATCC 30-2020 derivative:ATCC CRL-10741 derivative:ATCC CRL-11997 purified DNA:ATCC HB-8065D Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

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Cell Biology

ATCC® Number:	CRL-1764™ (Order this Item)
Designations:	Rat2
Depositors:	B Ahrens
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Rattus norvegicus (rat)
Morphology:	fibroblast
Source:	**Disease:** normal **Strain:** Fischer
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:	embryo; fetus
Comments:	This line was derived from the a 5-bromo-2'-deoxyuridine resistant strain of the Fischer rat fibroblast 3T3 like cell line, Rat1 (developed by W.C. Topp). Rat2 lacks detectable nuclear thymidine kinase, is highly transfectable by exogenous DNA and is phenotypically normal.
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%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:6 is recommended **Medium Renewal:** Twice per week Remove medium, and rinse with 0.25% trypsin, 0.03% 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 ce is detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	recommended serum:ATCC 30-2020 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

Price: $323.00

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- Material Transfer Agreement
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References:

1985. Topp WC. Normal rat cell lines deficient in nuclear thymidine kinase. Virology 113: 408-411, 1981. PubMed: 7269249
33037: Perg M, et al. Partial inhibition of Na+/K+-ATPase by ouabain induces the Ca2+-dependent expressions of early-response genes in cardiac myocytes. J. Biol. Chem. 271: 10372-10378, 1995. PubMed: 8626609

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Price: $264.00

ATCC® Number:	CCL-64™ (Order this Item)
Designations:	Mv 1 Lu (NBL-7)
Depositors:	AJ Kniazeff
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	Mustela vison (mink)
Morphology:	epithelial
Source:	Organ: lung
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: May, 1964
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	herpes simplex; reovirus 3; vaccinia; vesicular stomatitis (Ogden)
Virus Resistance:	adenovirus 5; coxsackievirus A9, B5; poliovirus 2
Reverse Transcript:	negative
Cytogenetic Analysis:	Both male and female diploid cells as well as pseudodiploid cells are present. Approximately 58% of the cells have a chromosome number within + or - 1 of the diploid and one dicentric chromosome is present in some cells of the population., Both male and female diploid cells as well as pseudodiploid cells are present. Approximately 58% of the cells have a chromosome number within + or - 1 of the diploid and one dicentric chromosome is present in some cells of the population.
Age:	near term fetus
Gender:	male and female mixed
Comments:	The Mv 1 Lu (NBL-7) cell line was initiated by A.J. Kniazeff, W.A. Nelson-Rees and N.B. Darby, Jr., in May, 1964, from trypsinized lungs of several nearly full-term, unsexed fetuses of the Aleutian mink.The cells are useful for focus forming assays for murine and feline sarcoma viruses [PubMed: 4366800].

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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:

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 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: Liquid nitrogen vapor temperature

Related Products:

recommended serum:ATCC 30-2020
purified DNA:ATCC CCL-64D
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 medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

References:

26211: Henderson IC, et al. Mink cell line Mv 1 Lu (CCL 64). Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology 60: 282-287, 1974. PubMed: 4366800
32364: Miller AD, Chen F. Retrovirus packaging cells based on 10A1 murine leukemia virus for production of vectors that use multiple receptors for cell entry. J. Virol. 70: 5564-5571, 1996. PubMed: 8764070
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
32691: Wang H, et al. Modulation of ecotropic murine retrovirus by N-linked glycosylation of the cell surface receptor/amino acid transporter. J. Virol. 70: 6884-6891, 1996. PubMed: 8794331
33048: Feng XH, Derynck R. Ligand-independent activation of transforming growth factor (TGF) beta-signaling pathways by heteromeric cytoplasmic domains of TGF-beta receptors. J. Biol. Chem. 271: 13123-13129, 1996. PubMed: 8662796

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ATCC® Number:	CRL-1772™ (Order this Item)
Designations:	C2C12
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	myoblast Photo
Source:	**Tissue:** muscle **Strain:** C3H **Cell Type:** myoblast;
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 a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903] The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953] Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23422] 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: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C

Price: $256.00

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Subculturing:

Protocol: IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT.
Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture.
Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

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.
   Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2.
6. Incubate cultures at 37°C.

Preservation:

Medium Renewal: Every two to three days
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
recommended serum:ATCC 30-2020

References:

22903: Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524
22953: Blau HM, et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846
23427: Katagiri T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage (published erratum appears in J Cell Biol 1995 Feb;128(4):following 713). J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324
28236: Chow YH, et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336
32828: Kessler PD, et al. Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064
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ATCC® Number:	CRL-1658™ Order this Item
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 (CO_2), 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.

Price: $256.00

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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

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ATCC® Number: CRL-1651™ (Order this Item)
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*
Morphology: fibroblast

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)
Virus Susceptibility: SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region
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_2), 5%
Temperature: 37.0°C

Price: $264.00

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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 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

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 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:

0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank's BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCl-70

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ATCC® Number:	CRL-1650™ (Order this Item)
Designations:	COS-1
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain PAPOVAVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast
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)
Virus Susceptibility:	SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region
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. The cells contain a single integrated copy of the complete early region of the SV40 genome.
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_2), 5% Temperature: 37.0°C

Price: $264.00

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Subculturing:

Protocol:

1. Remove and discard 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 5 to 10 min).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detatch. Cells that are difficult to detatch 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 1:4 to 1:8 is recommended
   Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: Liquid nitrogen vapor temperature

Related Products:

recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70
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 medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

1822: Gluzman Y. SV40-transformed simian cells support the replication of early SV40 mutants. Cell 23: 175-182, 1981. PubMed: 6260373
32348: Meansky LM. The mutation rate of human immunodeficiency virus type 1 is influenced by the vpr gene. Virology 222: 391-400, 1996. PubMed: 8806523
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32972: Bhattacharyya DK, et al. Involvement of arginine 120, glutamate 524, and tyrosine 355 in the binding of arachidonate and 2-phenylpropionic acid inhibitors to the cyclooxygenase active site of ovine prostaglandin endoperoxide H synthase-1. J. Biol. Chem. 271: 2179-2184, 1996. PubMed: 8567676
33048: Feng XH, Derynck R. Ligand-independent activation of transforming growth factor (TGF) beta signaling pathways by heteromeric cytoplasmic domains of TGF-beta receptors. J. Biol. Chem. 271: 13123-13129, 1996. PubMed: 8662796
33149: Wang LH, et al. Identification of thromboxane A2 synthase active site residues by molecular modeling-guided site-directed mutagenesis. J. Biol. Chem. 271: 19920-19925, 1996. PubMed: 8702713
33176: Almuela N, et al. Mapping the binding site pocket of the serotonin 5-hydroxytryptamine 2A receptor. J. Biol. Chem. 271: 14672-14675, 1996. PubMed: 8663249

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ATCC® Number:	CCL-34™ (Order this Item)
Designations:	MDCK (NBL-2)
Depositors:	S Madin, NB Darby
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Canis familiaris*
Morphology:	epithelial
Source:	**Organ:** kidney **Disease:** normal
Cellular Products:	keratin
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:** September, 1958
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Human Coxsackievirus B 5 Reovirus type 2 Adeno-associated virus 4 Vaccinia virus Vesicular stomatitis virus Adeno-associated virus 5 Human Coxsackievirus B 3 Human Coxsackievirus B 4 Human poliovirus 2
Reverse Transcript:	negative
Cytogenetic Analysis:	Polyploidy 0.2%. Two large submetacentric chromosomes noted, presumably X chromosomes, and one or two additional chromosomes with median or submedian centromeres.
Age:	adult
Gender:	female

Price: $264.00

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Comments: The MDCK cell line was derived from a kidney of an apparently normal adult female cocker spaniel, September, 1958, by S.H. Madin and N.B. Darby.The cells are positive for keratin by immunoperoxidase staining.MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its proteolytic products.

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 (CO_2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Rinse the cell layer twice 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.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

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

Related Products: recommended serum:ATCC 30-2020
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
parental cell line:ATCC CCL-34.2
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

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