

Modification Form for Permit BIO-UWO-0147

Permit Holder: Sung Kim

Approved Personnel

(Please stroke out any personnel to be removed)

Boram Ham
Anthony Bruni
Sarah Spanton
Andrew Martins
Soon-Duck Ha

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	P. aeurogenosa, S. typhimurium, actobacillus rhamnosus, E. coli EC1000, E.Coli BL21.	
Approved Cells	Human (primary), rodent (primary), human (established), THP-1, rodent (established), RAW 2649	HEK293, HepG2, CHO L929, CaCo2, U937 NPC-1 deficient, Fibroblast (GM00038)
Approved Use of Human Source Material	Blood (whole), PARF-CFP, mRFP-Rab7, mRFP-Rab5	
Approved GMO	pTRK830, pOR128, pTRK669	
Approved use of Animals	mice	
Approved Toxin(s)	cholera, diphtheria, CONT'D, Anthratoxin	

* 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: Apr 28, 2008

Date of Last Modification (if applicable): Oct 5, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

We found that cathepsin B is involved in autophagy flux and defects in the flux causes cell death. NPC-1 deficient cells are defective in autophagy flux. We will examine if this cell types are more sensitive to autophagy induced cell death. Other cell types are for control or to examine cell death induced by LPS or anthrax toxins.

Thanks

SK

* 1929 - mouse fibroblast cell line
per e-mail from Dr. Kimi Nov 10/09
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Cell Biology

ATCC® Number:	CRL-1573™	<input type="button" value="Order this Item"/>	Price:	\$256.00
Designations:	293 [HEK-293]			Related Links ▶
Depositors:	FL Graham			NCBI Entrez Search
<u>Biosafety Level:</u>	2 [CELLS CONTAIN ADENOVIRUS]			Cell Micrograph
Shipped:	frozen			Make a Deposit
Medium & Serum:	See Propagation			Frequently Asked Questions
Growth Properties:	adherent			Material Transfer Agreement
Organism:	<i>Homo sapiens</i> (human) epithelial			Technical Support
Morphology:	 PHOTO			Related Cell Culture Products
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) virucide 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

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]

Comments:

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]

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%.

Propagation:

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:

Cell Biology

ATCC® Number:	HB-8065™	Order this Item	Price:	\$264.00
Designations:	Hep G2			Related Links ▶
Depositors:	Wistar Institute			NCBI Entrez Search
<u>Biosafety Level:</u>	1			Cell Micrograph
Shipped:	frozen			Make a Deposit
Medium & Serum:	See Propagation			Frequently Asked Questions
Growth Properties:	adherent			Material Transfer Agreement
Organism:	<i>Homo sapiens</i> (human) epithelial			Technical Support
Morphology:				Related Cell Culture Products
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
 TH01: 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 gramoxone (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:

Cell Biology

ATCC® Number:	CCL-61™	Order this Item	Price:	\$256.00
Designations:	CHO-K1		Related Links ▶	
Depositors:	TT Puck		NCBI Entrez Search	
<u>Biosafety Level:</u>	1		Cell Micrograph	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	Cricetulus griseus (hamster, Chinese) epithelial-like		Technical Support	
Morphology:			Related Cell Culture Products	
Source:	Organ: ovary			
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: 1957			
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)			
Virus Susceptibility:	vesicular stomatitis (Indiana); Getah virus			
Virus Resistance:	poliovirus 2; modoc virus; Button Willow virus			
Reverse Transcript:	negative			
Cytogenetic Analysis:	Chromosome Frequency Distribution 50 Cells: 2n = 22. Stemline number is hypodiploid.			
Gender:	female			
Comments:	The CHO-K1 cell line was derived as a subclone from the parental CHO cell line initiated from a biopsy of an ovary of an adult Chinese hamster by T. T. Puck in 1957. [22224] The cells require proline in the medium for growth. [25976]			
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%. Temperature: 37.0°C			

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.

Subculturing:

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

Medium Renewal: Once or twice between subculture

Preservation:

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

Storage temperature: liquid nitrogen vapor phase

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

Related Products:

Recommended medium (without the additional supplements or serum described under [ATCC Medium](#)): [ATCC 30-2004](#)

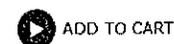
Cell Biology

ATCC® Number:	HTB-37™	<input type="button" value="Order this Item"/>	Price:	\$264.00
Designations:	Caco-2		Related Links ▶	
Depositors:	J Fogh		NCBI Entrez Search	
<u>Biosafety Level:</u>	1		Cell Micrograph	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	adherent		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human) epithelial		Technical Support	
Morphology:			Related Cell Culture Products	
Source:	Organ: colon Disease: colorectal adenocarcinoma keratin			
Cellular Products:	retinoic acid binding protein 1 retinol binding protein 2			
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 cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.			
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)			
Receptors:	heat stable enterotoxin (Sta, <i>E. coli</i>), expressed epidermal growth factor (EGF), expressed			
Virus Susceptibility:	Human immunodeficiency virus 1			
Tumorigenic:	Yes			
Reverse Transcript:	N			

Cytogenetic Analysis:	The stemline modal chromosome number is 96, occurring at 16% with polyploidy at 3.2%. Ten common markers were detected i.e., t(1q;?), 10q-, t(11q17q) and 7 others. The t(1q17q) and M11 were found in a portion of cells. The ins(2), 10q-, and t(15q;?) were generally paired, and t(11q;17q) and t(21q;?) were mostly three-copied. Normal N9 was absent, and N21 was lost in some cells. One to 4 small acrocentric chromosomes were detected. No Y chromosome with bright distal q-band was detected by Q-observation.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 1 PGM1, 1 PGM3, 1
Age:	72 years adult
Gender:	male
Ethnicity:	Caucasian
HeLa Markers:	N
Comments:	Upon reaching confluence, the cells express characteristics of enterocytic differentiation [PubMed ID: 1939345]. Caco-2 cells express retinoic acid binding protein I and retinol binding protein II [PubMed ID: 9040537].
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 20%. Atmosphere: air, 95%; carbon dioxide (CO ₂), 5% Temperature: 37.0°C
Subculturing:	

Catalog ID: **GM00038**

Product (Source): CELL CULTURE



- Overview
- Characterizations
- Phenotypic Data
- Publications
- External Links
- Images
- Protocols

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Blopsy Source Unspecified
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 9 YR
Sex Female
Race Black
Family 163 [View Pedigree](#)
Family Member 1
Relation to Proband daughter
Clinically Affected No
Confirmation Karyotypic analysis and Case history
Remarks 46,XX; 2% of cells show random chromosome loss and 4% show random chromosomal aberrations; skin biopsy; mother is GM000438
Catalog ID GM00038
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00
How to Order [Online Ordering](#)
 Assurance Form (Must have current form on file)
 Statement of Research Intent Form (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Passage Frozen 10

IDENTIFICATION OF SPECIES Species of Origin Confirmed by Nucleoside Phosphorylase, Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase
OF ORIGIN Isoenzyme Electrophoresis and by Chromosome Analysis

Phenotypic Data

Remark 46,XX; 2% of cells show random chromosome loss and 4% show random chromosomal aberrations; skin biopsy; mother is GM000438

Publications

Yamauchi Y, Reid PC, Sperry JB, Furukawa K, Takeya M, Chang CC, Chang TY, Plasma membrane rafts complete cholesterol synthesis by participating in retrograde movement of precursor sterols *The Journal of biological chemistry*282:34994-5004 2007

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Mirzayans R, Paterson MC, Waters R, Defective repair of a class of 4NQO-induced alkali-labile DNA lesions in xeroderma pigmentosum complementation group A fibroblasts. *Carcinogenesis* 6:555-9 1985

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External Links

dbSNP [dbSNP ID: 21465](#)
GEO [GEO Accession No: GSM88289](#)
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[GEO Accession No: GSM88291](#)
[GEO Accession No: GSM88307](#)
[GEO Accession No: GSM88308](#)
[GEO Accession No: GSM88309](#)

Images

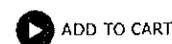
[View pedigree](#)

Protocols

Passage Frozen 10
Split Ratio 1:4
Temperature 37 C
Percent CO2 5%
Medium Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids
Serum 15% fetal bovine serum Not inactivated

Catalog ID: **GM03123**

Product (Source): CELL CULTURE



- Overview
- Characterizations
- Phenotypic Data
- Publications
- External Links
- Images
- Protocols

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Inherited Disorders
Class Disorders of Lipid Metabolism
Sample Description NIEMANN-PICK DISEASE, TYPE C1; NPC1
 NPC1 GENE; NPC1
Cell Type Fibroblast
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 9 YR
Sex Female
Race Caucasian
Family 451
Family Member 1
Relation to Proband proband
Clinically Affected Yes
Confirmation Biochemical characterization after cell line submission to CCR
Remarks See GM03124 Lymphoid; 38% of normal sphingomyelinase activity, normal B-galactosidase activity, and impaired cholesterol esterification in fibroblasts; the donor subject is a compound heterozygote; one allele carries a missense mutation C>T at nucleotide 709 (709C>T) in exon 6 of the NPC1 gene, resulting in a substitution of a serine for a proline at codon 237 [Pro237Ser (P237S)]; the second allele also carries a missense mutation T>C at nucleotide 3182 (3182T>C) in exon 21 which results in the substitution of a threonine for an isoleucine at codon 1061 [Ile1061Thr (I1061T)] in a transmembrane domain.

Catalog ID GM03123
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00
How to Order [Online Ordering](#)
 Assurance Form (Must have current form on file)
 Statement of Research Intent Form (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description NIEMANN-PICK DISEASE, TYPE C1; NPC1
 NPC1 GENE; NPC1
Passage Frozen 5

IDENTIFICATION OF SPECIES Species of Origin Confirmed by Nucleoside Phosphorylase, Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase
OF ORIGIN Isoenzyme Electrophoresis

sphingomyelin phosphodiesterase According to the submitter, biochemical test results for this subject showed decreased enzyme activity. EC Number: 3.1.4.12; 38% activity.

Gene NPC1
Chromosomal Location 18q11-q12
Allelic Variant 1 P237S; NIEMANN-PICK DISEASE, TYPE C1
Identified Mutation **PRO237SER**

Gene NPC1
Chromosomal Location 18q11-q12
Allelic Variant 2 607623.0010; NIEMANN-PICK DISEASE, TYPE C1
Identified Mutation **ILE1061THR**; In an initial study of 25 patients with type C1 Niemann-Pick disease, Millat et al. [Am. J. Hum. Genet. 65: 1321-1329 (1999)] identified a T-to-C transition at nucleotide 3182 of the NPC1 gene that led to an ile1061-to-thr substitution (I1061T) in 3 patients. The mutation, located in exon 21, affected a putative transmembrane domain of the protein. The mutation was particularly frequent in patients with NPC from western Europe, especially France and the U.K. and in Hispanic patients whose roots were in the Upper Rio Grande valley of the U.S. Millat et al. [Am. J. Hum. Genet. 65: 1321-1329 (1999)] concluded that the I1061T mutation originated in Europe and that the high frequency in northern Rio Grande Hispanics resulted from a founder effect.

Phenotypic Data

Remark See GM03124 Lymphoid; 38% of normal sphingomyelinase activity, normal B-galactosidase activity, and impaired cholesterol esterification in fibroblasts; the donor subject is a compound heterozygote; one allele carries a missense mutation C>T at nucleotide 709 (709C>T) in exon 6 of the NPC1 gene, resulting in a substitution of a serine for a proline at codon 237 [Pro237Ser (P237S)]; the second allele also carries a missense mutation T>C at nucleotide 3182 (3182T>C) in exon 21 which results in the substitution of a threonine for an isoleucine at codon 1061 [Ile1061Thr (I1061T)] in a transmembrane domain.

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External Links

dbSNP [dbSNP ID: 17690](#)
Gene Cards [NPC1](#)
Gene Ontology [GO:0004888 transmembrane receptor activity](#)
[GO:0005478 intracellular transporter activity](#)
[GO:0005624 membrane fraction](#)
[GO:0005764 lysosome](#)
[GO:0006886 intracellular protein transport](#)
[GO:0008158 hedgehog receptor activity](#)
[GO:0015248 sterol transporter activity](#)
[GO:0016021 integral to membrane](#)
[GO:0030301 cholesterol transport](#)
Locus Link [LocusLink ID: 4864](#)
OMIM [257220 NIEMANN-PICK DISEASE, TYPE C1; NPC1](#)
[607623 NPC1 GENE; NPC1](#)
Omim Description NIEMANN-PICK DISEASE WITH CHOLESTEROL ESTERIFICATION BLOCK
 NIEMANN-PICK DISEASE, CHRONIC NEURONOPATHIC FORM
 NIEMANN-PICK DISEASE, SUBACUTE JUVENILE FORM
 NIEMANN-PICK DISEASE, TYPE C; NPC
 NIEMANN-PICK DISEASE, TYPE C1; NPC1

Images

Data are not available

Protocols

Passage Frozen 5
Split Ratio 1:5
Temperature 37 C
Percent CO2 5%
Medium Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids
Serum 10% fetal bovine serum Not inactivated
Substrate None specified
Subcultivation Method trypsin-EDTA