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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 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 Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Dr. Murray Huff
DEPARTMENT	Medicine and Biochemistry
ADDRESS	Robarts Research Institute Room 4222
PHONE NUMBER	25793
EMERGENCY PHONE NUMBER(S)	519-432-6078
EMAIL	mhuff@uwo.ca

Location of experimental work to be carried out: Building(s) ___RRI_ Room(s)_4222, 4221, 4213, 4244C,4250, 4250C,4223, 3225, temporarily in 4217, if required for intermediate radioactive experiments roomO274

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, Heart and Stroke, Pfizer Canada Inc.
GRANT TITLE(S):
Regulation of Foam Cell Formation: Relationship to Atherosclerosis.
Regulation of ApoB Metabolism: Relationship to Atherosclerosis.
Regulation of hepatic lipoprotein secretion and glucose homeostasis by the citrus flavonoid nobiletin: Relationship to Atherosclerosis.

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Cindy Sawyez	csawyez@uwo.ca	Feb 8&9, 2005
Jane Edwards	jvedward@uwo.ca	Nov 3,2006

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

The use of these agents and substances will follow the UWO Biosafety guidelines and procedures manual. Laboratory personal protection is worn at all times.

The cell culture lines are used in a dedicated tissue culture room for containment level 2 in biological safety cabinets in RRI room 4244C and the frozen stocks are kept in liquid nitrogen in RRI room 4250C. Any waste plasticware, gloves or glass generated in the tissue culture process is autoclaved at RRI. Any media is added to bleach so the final concentration is at least 10% and left to sit for at least 1 hour prior to disposal down the drain with water.

Any blood handling is done using universal precautions in RRI room 4221 in a biological safety cabinet. Any short term storage of material is in containment level 2 rooms. Blood products and plasticware, gloves or glass that has been in contact with blood is incinerated by UWO.

Bacterial cultures will be grown in RRI rooms 4250 and 3225, collected for DNA extraction in RRI room 4222 and stored as frozen stocks at -80C in RRI room 4223 or as cultures on culture plates in RRI room 4217. Any bacterial liquid waste will be incubated in bleach before disposal. Plasticware or disposable items will be autoclaved before disposal.

The pertussin toxin is stored in RRI room 4244C in a labeled locked container. Any powder will be used in a fume hood to avoid breathing any compound. In solution it is used in RRI room 4244C. Any waste products will be packaged up for UWO lab waste disposal.

See following sheets for the summary proposals of the grants.

represents a central event in atherosclerosis. Foam cells arise from the dysregulation of signals governing the uptake of lipoproteins and those regulating cholesterol efflux to extracellular acceptors. Although some mechanisms responsible for lipoprotein uptake and cholesterol efflux have been characterized, the identification of all relevant pathways and their coordination have not been fully elucidated. This renewal is focused on characterizing novel strategies for the prevention of foam cell formation induced by the atherogenic lipoproteins, human VLDL and VLDL remnants (REM), in both their native and oxidized forms, and builds on recent discoveries implicating nuclear hormone receptors as the master regulators of cellular cholesterol homeostasis.

Hypothesis: Induction of macrophage and SMC foam cell formation by native or oxidized VLDL and VLDL-REM can be attenuated through regulating the activation of nuclear hormone receptors.

In this proposal we will exploit 3 significant observations and address the following specific questions:

PART 1: In macrophages, does enhanced endogenous synthesis of 24(S),25-epoxycholesterol inhibit foam cell formation induced by oxVLDL/VLDL-REM? What mechanisms are involved?

We discovered in macrophages that increased synthesis of 24(S),25-epoxycholesterol, a potent regulatory oxysterol, can be induced through partial inhibition of the enzyme oxido-squalene lanosterol cyclase (OSC). The resultant dramatic reduction of macrophage cholesteryl ester (CE) accumulation in cells challenged with native VLDL occurs via dual mechanisms: down-regulation of LDL-receptor expression and increased expression of ABCA1- and ABCG1-mediated cholesterol efflux. We now propose to determine if these effects are mediated through the oxysterol-sensitive transcription factors, SREBP-1, SREBP-2 or LXR. Furthermore, we will establish whether this strategy is effective for inhibition of oxVLDL/oxVLDL-REM-induced CE accumulation in macrophages. The only known biological functions of 24(S),25-epoxycholesterol are those involving the regulation of cholesterol and lipid metabolism. In order to expose novel biological roles for this oxysterol in foam cell formation, gene expression profiling using Affymetrix microarrays in control and OSC inhibited cells are proposed.

PART 2: In macrophages, does inhibition of HMG-CoA reductase upregulate cholesterol efflux and attenuate native and oxidized VLDL/VLDL-REM induced foam cell formation? What mechanisms are involved?

We have discovered that inhibition of HMG-CoA reductase blocks VLDL/VLDL-REM-induced macrophage foam cell formation. Unexpectedly, this was due to decreased expression of the LDL-receptor and increased ABCA1/G1 expression. We believe that this effect is independent of cellular cholesterol and involves decreased geranylgeranylation of the small GTPase RhoA, which in turn alters the activity of PPARγ, PPARα, LXR and SREBP-2. We will determine (1) If CE accumulation induced by oxVLDL/oxVLDL-REM is also inhibited;(2) the role of geranylgeranyl pyrophosphate; (3) the role of RhoA or RhoA associated kinases (ROCK I and II), and (4) the role of LXR and SREBP-2.

PART 3: In SMCs, can CE accumulation, induced by VLDL/VLDL-REM, be attenuated by enhancing cholesterol efflux? Can this be achieved through activation of PPARγ, synthesis of 24(S),25-epoxycholesterol or inhibition of HMG-CoA reductase? Do the mechanisms involved differ from macrophages?

We made the intriguing observation that two distinct human SMC subpopulations are differentially converted to foam cells. The clone that was larger, spindle-shaped, slow growing and less growth factor responsive, preferentially accumulated CE when incubated with native or oxidized VLDL. We propose to identify the mechanisms involved, specifically: (1) Can VLDL/VLDL-REM induced CE accumulation be inhibited by PPARγ or PPARα ligands, endogenous 24(S),25-epoxycholesterol or HMG-CoA reductase inhibition? (2) Does LXR-mediated ABCA1/G1 upregulation lead to enhanced cholesterol efflux? (3) Are the LDL-receptor or scavenger receptors involved?

Hepatic overproduction and/or delayed catabolism of the atherogenic, apolipoprotein B (apoB)-containing lipoproteins are core features of dyslipidemias linked to premature atherosclerosis. The pertinent human phenotypes include combined hyperlipidemia and dyslipidemia of the metabolic syndrome, as characterized by elevations in plasma triglyceride (TG), and very low density lipoprotein (VLDL)-apoB, decreased high density lipoprotein (HDL), glucose intolerance and insulin resistance. Increased free fatty acid flux, stimulation of hepatic TG synthesis by hyperinsulinemia, and the failure of insulin to both inhibit VLDL-apoB secretion and upregulate the low density lipoprotein receptor (LDLr) are thought to contribute mechanistically to the dyslipidemia of insulin resistance. Although some mechanisms responsible for maintaining normal hepatic lipid metabolism have been characterized, the components and co-ordination of relevant pathways remain incompletely understood. Furthermore, few available therapeutic strategies effectively correct the insulin resistance and associated disturbances in plasma lipids. This proposal focuses on characterizing novel strategies to inhibit hepatic apoB secretion, to decrease atherogenic plasma lipids, to correct the perturbed glucose metabolism associated with insulin resistance, and ultimately prevent atherosclerosis.

Hypothesis: Flavonoids decrease secretion of hepatic apoB-containing lipoproteins by activating signal transduction pathways, thus attenuating dyslipidemia, hepatic steatosis, insulin resistance and atherosclerosis.

Part 1: Do naringenin and nobiletin activate cell signalling pathways through FGF21 to inhibit apoB secretion and attenuate dyslipidemia, hepatic steatosis, insulin resistance and atherosclerosis? We discovered that in *Ldlr*$^{-/-}$ mice fed a high-fat diet, naringenin and nobiletin normalized VLDL-apoB secretion, dyslipidemia, hepatic steatosis, hyperinsulinemia and importantly, protected against atherosclerosis. The effect of naringenin involved prevention of SREBP1c-mediated lipogenesis in liver and increased hepatic β-oxidation through a PGC1α/PPARα-mediated transcription program. Fibroblast growth factor 21 (FGF21) is a recently discovered metabolic regulator that requires activation by PGC1α/PPARα. Therefore, using cultured hepatocytes and *Fgf21*$^{-/-}$ mice, we will determine if naringenin and nobiletin stimulate β-oxidation and decrease apoB secretion through a FGF21-dependent pathway.

Part 2: In mice, do naringenin and/or related flavonoids inhibit hepatic apoB secretion through activation of PI3K/Akt and downstream signalling through FoxO1, GSK3β or mTOR? We discovered that naringenin and nobiletin, like insulin, inhibit hepatocyte apoB secretion by activating PI3K/Akt and MAPK^{erk} to increase LDLr expression and inhibit microsomal triglyceride transfer protein expression, respectively. Of significance, naringenin and nobiletin do not signal through the insulin receptor, and both flavonoids sensitize hepatocytes to inhibition of apoB secretion by insulin. We will identify, in mice, the downstream signalling pathways through which hepatic fatty acid synthesis and apoB secretion are regulated by these flavonoids.

Part 3: Do naringenin and nobiletin attenuate dyslipidemia, hepatic steatosis, insulin resistance and atherosclerosis in a genetic model of severe insulin resistance: the *ob/ob* mouse? Protection by flavonoids may only occur in mice fed a high fat diet with competent leptin signalling. Therefore, their efficacy will be examined in leptin-deficient *ob/ob* mice fed a low fat diet.

Part 4: Do naringenin and nobiletin enhance the effect of insulin in mice fed a low fat diet? *In vitro*, flavonoids sensitize hepatocytes to the pluripotential effects of insulin. The ability of flavonoids to amplify insulin action and regulate hepatic lipid metabolism will be evaluated in normal mice.

Part 5: In mice, do naringenin and nobiletin reverse established dyslipidemia, hepatic steatosis, hyperinsulinemia and atherosclerosis? From a therapeutic perspective, it is important to evaluate if the flavonoids can correct pre-existing metabolic abnormalities and atherosclerosis.

Regulation of hepatic lipoprotein secretion and glucose homeostasis by the citrus flavonoid nobiletin: Relationship to Atherosclerosis

Hepatic overproduction and/or delayed catabolism of atherogenic lipoproteins are characteristic of the dyslipidemia associated with insulin resistance. The inability of insulin to both inhibit hepatic VLDL apoB secretion and upregulate hepatic LDL receptors (LDLr) is thought to underlie the dyslipidemia of insulin resistance, and is strongly linked to atherogenesis. Insulin inhibits apoB100 secretion from hepatocytes, through activation of phosphatidylinositol-3-kinase (PI3K) and mitogen activated protein kinase/extracellular regulated kinase ($MAPK^{erk}$) signalling. In cultured hepatocytes, we discovered that the polymethoxylated citrus flavonoids, nobiletin activates insulin signalling pathways. In hypercholesterolemic, hyperinsulinemic hamsters, others have shown that a mixture of polymethoxylated flavonoids containing nobiletin normalized plasma triglycerides and glucose, suggesting nobiletin has insulin-like properties. This proposal will test the hypothesis that nobiletin regulates hepatic lipid and glucose metabolism through activation of insulin signalling cascades, and attenuates atherosclerosis. Preliminary studies in hepatocytes demonstrate that nobiletin significantly inhibits apoB100 secretion, increases LDLr expression and suppresses microsomal triglyceride transfer protein (MTP) expression through activation of $MAPK^{erk}$ signalling. However, nobiletin, unlike insulin, does stimulate lipogenesis. Three objectives will be addressed: (1) To fully elucidate the mechanism by which nobiletin inhibits VLDL apoB100 secretion from hepatocytes; (2) To determine if nobiletin regulates the expression and activity of enzymes rate-limiting in the metabolism of glucose in hepatocytes and determine the signalling mechanism(s) involved and (3) To determine if nobiletin attenuates the dyslipidemia, hyperglycemia, and atherosclerosis in a mouse model of insulin resistance. These experiments have the potential to provide novel strategies for dyslipidemic states, including the dyslipidemia of insulin resistance.

1.1 Does your work involve the use of biological agents? • YES O NO
non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? 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	1	Invitrogen	• 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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	See attached sheet	Not applicable
Rodent	• Yes O No	See attached sheet	

	in your work?		
Human	• Yes O No	See attached sheet	
Rodent	• Yes O No	See attached sheet	
Non-human primate	O Yes • No		
Other (specify)	O Yes • No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material 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	Hospital lipoprotein clinic samples & volunteers	O Yes • Unknown		O 1 • 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid	Lipoproteins isolated from human blood samples	O Yes • Unknown		O 1 • 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	NA	O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)	NA	Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

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

Vector Construction			Transduced	that results from transduction
NA				

* 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 • YES O NO
- E1A oncogene • YES O NO
- Known oncogenes O YES, please specify _____ • NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ • NO

4.5 Will virus be replication defective? • YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES • NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used____mouse C57BL/6, LDLR-/-, fgf21-/-_____

6.3 AUS protocol # ___2008-071_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: • NO

be used (see list below)? O YES • No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)____pertussis toxin_____
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___18ug/kg IP in mice, 114µg/kg in rat_____

8.4 How much of the toxin is handled at one time*? _____1µg_____

8.5 How much of the toxin is stored*? 50µg is the smallest quantity to buy. It is made up and aliquoted 1ug per tube. Only 1 tube is used at any time.

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ • NO

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

9.0 Insects

9.1 Do you use insects? 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:

_____

_____

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 YES, Please attach the CFIA permit & 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_____ • NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ • NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

HC or CFIA Containment Level required. O 1 • 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

• YES, permit # if on-campus__BIO-RRI-0008_____
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 Myung Hyuk Date: Nov 23, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____will follow containment level 2 _____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

If there is a needlestick injury the wound would be expressed to bleed, then washed with soap and water, and OHS will be contacted.

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Ronald Noseworthy
Date: November 29, 2010

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

Containment levels are indicated in brackets after the cell name.

9D9 (level 1) Hybridoma-fuse P3X63-Ag8.653 mouse myeloma cells with spleen lymphocytes from a BALB/c mouse, secrete anti rabbit LDL receptor antibody(ATCC #CRL-1703)
AC29 (treated as level 2) Chinese hamster ovary cell line +/- human ACAT 1&2
C7 (level1) Hybridoma-fuse Sp2/0-Ag14 cells with spleen lymphocytes from a BALB/c mouse, secrete anti LDL receptor antibody(ATCC #CRL-1691)
HEK 293 (level 2) human kidney cell line, contains EIA oncogene(ATCC #CRL-1573)
HepG2 (level 1) human liver cell line(ATCC #HB-8065)
Human primary fibroblasts (level2) Coriell GM02036(normal cells)
Coriell GM03123(NPC1-/- cells)
Human primary monocyte derived macrophages(level 2) isolated in room 4221
Human primary smooth muscle cells(level 2) isolated in Dr. Pickering's lab(A2, B5 and C6)
IgG-1D2 (level 1) hybridoma-spleen cells were fused with Sp2/0-Ag14 myeloma cells, secrete human SREBP-2 antibody (ATCC #CRL-2545)
J774A.1 (level 1) mouse macrophage cell line(ATCC #TIB-67)
McA-RH7777 (level1) rat hepatoma cell line(ATCC #CRL-1601)
Also RH-7777 lines transformed with 17, 23, 48, and 100% of human apoB
MCF7 (level 1) human breast cancer cells(ERalpha positive)(ATCC #HTB-22)
MDA-MB-231 (level 1) human breast cancer cells(ER negative)(ATCC #HTB-26)
Mouse primary hepatocytes (level 1) C57BL/6, LDLR-/- and fgf21-/- mice
Mouse primary macrophages (level 1) C57BL/6, LDLR-/-, and fgf21-/- mice
PU5 (level 1) mouse monocyte-macrophage cell line(ATCC #TIB-61)
THP-1 (level 1) human monocyte cell line(ATCC #TIB-202)

Bacteria:
DH5α(level 1) competent E. Coli obtained Invitrogen #18265017

Vectors:(SV40 promoter and herpes simplex)
pcDNA3 from Invitrogen
pcDNA3.1/*myc*-His from Invitrogen
pEYFP-C1 from Clontech
pRL-TK from Promega
pTK-Luc from Clontech
pSG5 from Stratagene
pRS from OriGene

4.2

Bacteria Used for Cloning *	**Plasmid(s) ****	**Source of Plasmid**	**Gene Transfected**	**Describe the change that results from transformation or tranfection**
E. Coli DH5α	pcDNA3 and pcDNA3.1/*myc*-His	Invitrogen	Human Estrogen receptor alpha	-when transfected into mammalian cells, production of ERα with or without a Histidine tag
E. Coli DH5α	pEYFP-C1	Clonetech	Human *Ras* Dominant negative or constitutively active	-when transfected into mammalian cells will produce Ras protein labeled with yellow fluorescent protein
E. Coli DH5α	pRL-TK	Promega	*Renilla* Luciferase	-produces *Renilla* luciferase in transfected mammalian cells.
E. Coli DH5α	pTK-Luc	Clonetech	Response elements from *LXR, PPAR, ERα* upstream from firefly luciferase gene	-in transfected mammalian cells, will produce luciferase protein from *Photinus Pyralis* if RE is activated.
E. Coli DH5α	pSG5	Stratagene	Mouse *Pparα*, *Pparγ1*, *Pparγ2*, *Pparδ*. Human *PPARα*, *PPARγ1*, *PPARγ2* and *PPARδ*.	-expression of receptors in transfected mammalian cells.
E. Coli DH5α	pRS	OriGene	Short hairpin RNA specifically directed against human *ABCG1*	-expression of shRNA sequence (see next section below)
				.

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I
Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
Physical data

Pink or red aqueous liquid

Info on Cell Line(s)

SECTION III
Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV
Fire and explosion

Not applicable

SECTION V
Reactivity data

Stable. Hazardous polymerization will not occur.

SECTION VI
Method of disposal

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII
Special protection information

For Biosafety Level 1 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII
Special precautions or comments

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

Product Description

Print this Page

Cell Biology

ATCC® Number: CRL-1703™ Order this Item

Price: $399.00

Designations: 9D9

Depositors: JL Goldstein

Isotype: IgG1

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension

Organism: Mus musculus (B cell); Mus musculus (myeloma) (mouse (B cell); mouse (myeloma))

Morphology: lymphoblast

Source: **Cell Type: hybridoma:** B lymphocyte;

Cellular Products: immunoglobulin; monoclonal antibody; against bovine low density lipoprotein (LDL) receptor

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.

Antigen Expression: H-2d

Comments: Mice were immunized with trypsin digested bovine adrenal LDL receptor.
Spleen cells were fused with P3X63Ag8.653 myeloma cells.
The antibody reacts with LDL receptors of normal rabbits but not with those of Watanabe Heritable Hyperlipidemic rabbits.
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%.

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normal and Watanabe Heritable Hyperlipidemic rabbits. J. Clin. Invest. 74: 1017-1026, 1984. PubMed: 6088578
23273: Kita T, et al. Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia. Proc. Natl. Acad. Sci. USA 78: 2268-2272, 1981. PubMed: 6264472

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Abstract. We have previously reported the isolation of Chinese hamster ovary cell mutants deficient in acyl-coenzyme A/cholesterol acyltransferase (ACAT) activity (Cadigan, K. M., J. G. Heider, and T. Y. Chang. 1988. *J. Biol. Chem.* 263:274–282). We now describe a procedure for isolating cells from these mutants that have regained the ability to synthesize cholesterol esters. The protocol uses the fluorescent stain Nile red, which is specific for neutral lipids such as cholesterol ester. After ACAT mutant populations were subjected to chemical mutagenesis or transfected with human fibroblast whole genomic DNA, two revertants and one primary transformant were isolated by virtue of their higher fluorescent intensities using flow cytofluorimetry. Both the revertants and transformant have regained large amounts of intracellular cholesterol ester and ACAT activity. However, heat inactivation experiments revealed that the enzyme activity of the transformant had heat stability properties identical to that of human fibroblasts, while the ACAT activities of the revertants were similar to that of other Chinese hamster ovary cell lines. These results suggest that the molecular lesion in the ACAT mutants resides in the structural gene for the enzyme, and the transformant has corrected this defect by acquiring and stably expressing a human gene encoding the ACAT polypeptide. Secondary transformants were isolated by transfection of ACAT mutant cells with primary transformant genomic DNA. Genomic Southern analysis of the secondary transformants using a probe specific for human DNA revealed several distinct restriction fragments common to all the transformants which most likely comprise part or all of the human ACAT gene. The cell lines described here should facilitate the cloning of the gene encoding the human ACAT enzyme.

ACYL-coenzyme A/cholesterol acyltransferase (ACAT)[1] is an intracellular enzyme that uses cholesterol and fatty acyl-coenzyme A (CoA) to form cholesterol esters (10, 50). The enzyme is localized to the rough endoplasmic reticulum in rat liver (2, 24); is highly regulated in many cell types and tissues; and is believed to play an important role in cholesterol metabolism in various cells and tissues such as the small intestinal mucosa, hepatocytes, and the steroid hormone–producing tissues (10, 50).

Although ACAT has been studied intensively, little is known about its molecular structure. In rat liver, the active site of the enzyme has been localized to the cytoplasmic surface of the microsomal vesicles using a combination of detergent and protease treatments (24, 34), but whether the enzyme spans the entire membrane could not be determined. Recent chemical modification studies have demonstrated that an essential histidyl and sulfhydryl residue(s) may reside at or near the active site of the enzyme. ACAT activities from different rabbit tissues have different sensitivities to the histidyl-modifying reagents, suggesting the existence of different ACAT subtypes (31, 32).

1. *Abbreviations used in this paper*: ACAT, acyl-coenzyme A/cholesterol acyltransferase; CHO, Chinese hamster ovary; CoA, coenzyme A; LDL, low density lipoprotein.

ACAT activity has been solubilized and reconstituted from various cultured cells (4, 17, 29), rat liver (51), and pig liver (16). Although these procedures have allowed enzyme activity to be measured in a defined lipid environment, little progress has been made in purifying the solubilized preparations. Partially purified ACAT fractions that contain up to 100-fold higher enzyme-specific activity than unfractionated pig liver microsomes (16) still contain numerous protein bands when analyzed by gel electrophoresis (unpublished results from this laboratory). The gene(s) encoding this enzyme has not been isolated and no antibodies directed against ACAT have been reported.

Chinese hamster ovary (CHO) cells are a fibroblast-like cell line in which cholesterol ester synthesis is highly regulated by exogenous sources of cholesterol, such as low density lipoprotein (LDL) (8, 17, 33), and by endogenous cholesterol synthesis (7). This laboratory recently reported the isolation of CHO cell mutants almost entirely lacking ACAT activity (5). All of the isolated mutants belonged to the same complementation group and possessed a defect in the ACAT enzyme itself or in a factor needed for production of the enzyme. We now report a procedure for isolating revertants that have regained enzyme activity. The selection uses the

enzyme-deficient mutants with human high molecular weight DNA. A combination of biochemical and DNA hybridization experiments strongly suggests that the transformants have acquired and express a human ACAT structural gene. The results reported here should facilitate the isolation and cloning of the human ACAT gene, which should prove invaluable in efforts to understand the molecular structure and regulation of this enzyme.

Materials and Methods

Reagents

Oleyl-CoA was synthesized as described by Stadtman using oleyl anhydride (49) and [^{3}H]oleyl-CoA was synthesized by another method (3). Quantitation of the oleyl-CoA preparations were made assuming an extinction coefficient at 260 nm of 15.4 mM^{-1} cm^{-1} (57). Purity was judged as 98% by TLC analysis in a solvent system of butanol/acetic acid/water (5:2:3) and by measuring the A_{232}/A_{260} ratio (49). Nile red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) was purified from Nile blue (Sigma Chemical Co., St. Louis, MO) by the method of Thorpe (53) as modified by Greenspan et al. (23). Concentrated stocks were made in ethanol and stored at 4°C protected from light. Compound 58-035 (3-[decyldimethylsilyl]-*N*-[2-(4-methylphenyl)-1-phenylethyl]propanamide) was provided by Dr. John Heider (Sandoz Inc., East Hanover, NJ). Mevinolin was a gift from Alfred Alberts (Merck & Co., Inc., Rahway, NJ). Both of these compounds were added to the culture medium from a concentrated dimethyl sulfoxide stock. pSV2-neo in *Escherichia coli* strain HB101 was a generous gift from Dr. Peter Southern (Research Institute of Scripps Clinic, La Jolla, CA) and pBLUR8 in HB101 was provided by Dr. Joanne Zurlo (Dartmouth Medical School, Hanover, NH) with permission from Dr. Warren Jelinek (New York University Medical Center, New York). The chloroform, methanol, and isopropanol used for the cholesterol analysis were from Mallinckrodt Inc. (Paris, KY) or Fisher Scientific Co. (Pittsburgh, PA) and were nanograde and spectranalyzed grade, respectively. Cholesterol oxidase was generously provided by Dr. Albert Chen (Beckman Instruments, Inc., Fullerton, CA). Horseradish peroxidase (P-6140), cholesterol esterase (C-1892), phosphatidylcholine type XI (P-2772), and all other enzymes and biochemical reagents were from Sigma Chemical Co. Other organic solvents and chemicals were from Fisher Scientific Co. and were of reagent grade quality.

Cell Culture

A primary culture of human fibroblasts was obtained from the foreskin of a healthy newborn. The tissue was dissociated using bacterial collagenase and trypsin as described by Dayer et al. (15) and the culture was used between the seventh and fifteenth passage. Human fibroblasts and CHO cell lines were grown as monolayers; human fibroblasts in MEM (Gibco Laboratories, Grand Island, NY) supplemented with 2 mM glutamine; and CHO cells in F-12 medium minus linoleic acid. Both media were supplemented with antibiotics as previously described (4, 5) and 10% FCS (Sigma Chemical Co.). When delipidated FCS was used, it was prepared according to a published procedure (6) as modified by Chin and Chang (14). Human LDL (d = 1.019–1.063 g/ml) was prepared from human plasma by sequential flotation in the presence of protease inhibitors as previously described (5).

Mutagenesis and DNA Transfections

The ACAT mutant cell line, AC29, was mutagenized with 125 μg/ml *N*-nitroso-*N*-ethylurea as previously described (5). Cotransfections of AC29 with the plasmid pSV2-neo, which confers resistance to the cytotoxic drug G418 (48), and human high molecular weight DNA were carried out as follows. High molecular weight DNA was prepared from cultured cells according to a published procedure (56). AC29 cells were transfected by the calcium phosphate coprecipitation technique of Graham and van der Eb (21) as modified by Wigler et al. (55) except that the precipitate was left on the monolayers for 12 h. During the course of this work, it was found that the frequency of G418-resistant colonies obtained by transfection could be in-

μg/ml G418 (Gibco Laboratories). Medium was replaced every other day for 1 wk, and then the cells were allowed to grow for 1 wk more without a change of medium. The resistant colonies were then pooled and maintained at 100 μg/ml G418 while the brightest Nile red-stained cells were isolated.

Isolation of Nile Red–positive Cells

All solutions used in preparing the cells for the cytofluorograph and fluorescence microscopy were in sterile Hank's balanced salt solution containing no phenol red. Confluent monolayers were washed three times and incubated with 0.003% trypsin for 15–20 min at 37°C. Cells were transferred to a sterile tube containing 1/10 vol of 0.075% soybean trypsin inhibitor (Sigma Chemical Co. T-9253) and then 2 vol of 150 ng/ml Nile red (final ethanol concentration, 0.2%) was added. The cells were gently mixed and allowed to sit for 15 min at room temperature protected from light. The stained cells were analyzed for green fluorescence (excitation wavelength 488 nm; emission wavelength 515–530 nm) using the Ortho Diagnostic Systems, Inc. (Raritan, NJ) cytofluorograph system 50H with the brightest 0.1% or 0.2% cells sorted into plates containing medium without sodium bicarbonate (to keep the pH from becoming too alkaline). The media containing the sorted cells were diluted two- to threefold with media containing bicarbonate and placed in a 5% CO_2 incubator at 37°C. The next day the medium was replaced with bicarbonate-containing medium. After 10 d colonies were visible and could be prepared for another round of cell sorting or examined under a low power phase microscopy for the presence of intracellular lipid droplets.

Fluorescence Microscopy

Cells were grown on glass cover slips. To prepare for viewing, the cover slips were rinsed five times and then stained for 6 min at room temperature with 100 ng/ml Nile red. After staining, the coverslips were rinsed, mounted onto slide chambers, and kept moist with the salt solution. Immediately thereafter, the cells were viewed using a Carl Zeiss, Inc. (Thornwood, NY) universal microscope and a 67× achromat oil-immersion phase-contrast objective (Carl Zeiss Inc.) with differential-interference contrast or epifluorescence (excitation 485 ± 10 nm; emission 520–560 nm) optics. Paired micrographs were taken with TMAX-400 film (Eastman Kodak Co., Rochester, NY) and developed according to instructions given by the manufacturer.

Sterol Analysis

Cells were grown in 25-cm^2 flasks and harvested in 1 ml 0.2 M NaOH as described previously (5). The NaOH sample was neutralized by adding HCl and phosphate buffer (8) and then Folch extracted (19). After drying under N_2, the samples were resuspended in 1 ml isopropanol and aliquots (30 or 60 μl) were taken for cholesterol determination using a fluorometric procedure (25) either with or without cholesterol esterase preincubation. Control experiments using radiolabeled cholesterol revealed recoveries of between 90 and 100% after extraction. The procedure for preparing the samples described above differs from the one previously used by this laboratory (5). In the previous procedure, aliquots were taken from the NaOH sample, extracted, dried, and resuspended in a small volume of isopropanol. The effect of the two different protocols upon the values obtained is discussed in the Results section.

ACAT Assays

For the [^{3}H]oleate pulse, all F-12 media were supplemented with 1.5 mM $CaCl_2$ because F-12 medium is low in calcium (0.3 mM) and the binding of LDL to its receptor is calcium dependent (20). The monolayers were pulsed with a [^{3}H]oleate/BSA solution and analyzed for incorporation of radiolabel into cholesterol oleate as previously described (5, 8) except that the blank was determined and subtracted from the reported values by pulsing AC29 cells grown in the presence of 58-035, a specific inhibitor of ACAT (43). The blank value was between 1.2 and 2.0 pmol/min per mg. For the in vitro ACAT assays, cell homogenates were prepared by the hypotonic-shock and scraping method (12) and used immediately. The microsomal assay has been described in detail previously (5, 8, 17). The reconstituted ACAT assay was performed as described in Cadigan and Chang (4). During

the lot previously used (46F-8430; these results can be found by comparing activities reported in Figs. 5 and 6). TLC analysis revealed no detectable contaminants in the two lots. This phenomenon is currently being pursued in this laboratory and will not be discussed further in this report. In all three assays described above, control experiments using [^{14}C]cholesterol oleate as a standard revealed recoveries of 70–77% after extraction and TLC. All protein determinations were made using the Peterson modification (40) of the method of Lowry et al. (36); no TCA precipitation was performed on the NaOH cell extracts in the sterol analysis and the [^{3}H]oleate pulse.

Southern Analysis

Whole genomic DNA samples were digested with restriction enzymes (15 U/μg DNA) for 36 h at 37°C. The digested samples were run on an 0.8% agarose gel and transferred to nylon filters (ICN Laboratories, Inc., Plainview, NY) by the method of Southern (47) as modified by Reed and Mann (41). Filters were prehybridized in a solution containing 25 mM KPO_4, pH 7.4, 5× SSC, 5× Denhart's solution, 100 μg/ml sonicated and denatured salmon sperm DNA, 50% formamide, and 1% SDS for 12 h at 42°C and then incubated with an identical solution containing 10% dextran sulfate and the denatured ^{32}P-probe. The 300-bp Bam HI fragment of the plasmid pBLUR8, which contains a human repetitive element of the Alu family (44), was used as the probe. The plasmid was digested with Bam HI and the Alu-containing fragment was excised from a low melting agarose gel and radiolabeled by the oligolabeling method of Feinberg and Vogelstein (18). After incubation in the hybridization buffer for 48 h at 42°C, filters were washed two times in 2× SSC/0.1% SDS supplemented with 0.05× bovine lacto transfer technique optimizer (27), followed by a 0.1× SSC/0.1% SDS wash, both at room temperature, before a final wash in 0.1× SSC/0.1% SDS at 55°C for 60 min. The filters were air dried and exposed to Kodak X-OMAT AR film with a Dupont Co. (Wilmington, DE) Lightning Plus intensifying screen for 3–5 d before developing.

Nile red is a highly fluorescent compound which preferentially partitions into hydrophobic environments such as intracellular neutral lipid droplets (22, 23). The Nile red fluorescent patterns of the ACAT mutant, AC29, and its parental cell line, 25-RA, were compared. 25-RA was isolated from wild-type CHO cells by its resistance to the cytotoxic effects of 25-hydroxycholesterol (11). Unlike wild-type cells, the uptake of LDL and the rate of endogenous cholesterol synthesis in 25-RA cells are partially resistant to suppression by exogenous sterols (5, 11). This results in an elevated rate of cholesterol ester synthesis leading to a large accumulation of intracellular cholesterol ester (5, 9). In contrast, the mutant cell line AC29 contains <1% of the ACAT activity of 25-RA and has greatly reduced intracellular cholesterol ester (5).

As shown in Fig. 1, there is a clear difference in the appearance of 25-RA and AC29 cells when viewed with differential-interference contrast (Fig. 1, *a* and *c*) and epifluorescence (Fig. 1, *b* and *d*) optics. 25-RA contained numerous birefringent perinuclear particles (Fig. 1 *a*) which were brightly stained with Nile red (Fig. 1 *b*). These particles were not found in AC29 cells, or in 25-RA cells grown in the presence of the specific ACAT inhibitor, 58-035 (data not shown). The light, diffuse fluorescence seen in AC29 cells (Fig. 1 *d*) is seen in all CHO cells examined thus far that lack cholesterol ester. The filters used for the fluorescent micrographs in Fig. 1 allowed only green fluorescence to be seen (520–560 nm).

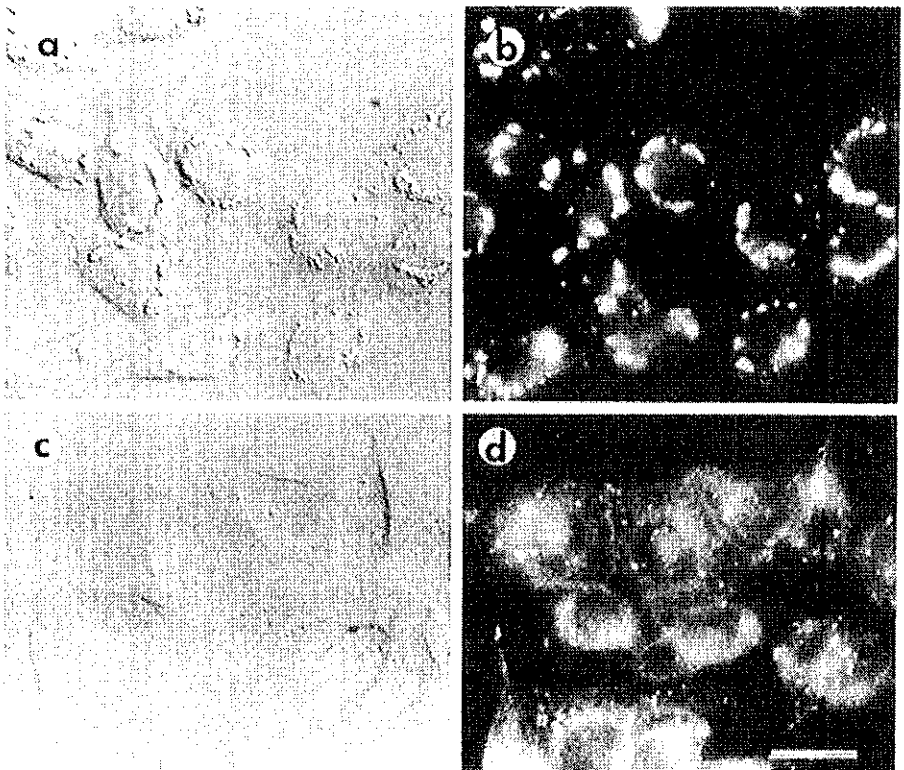

Figure 1. Nile red–stained 25-RA and AC29 cells viewed with differential-interference contrast or fluorescence microscopy. 25-RA (*a* and *b*) and AC29 (*c* and *d*) were plated at a density of 6.5 × 10^4 cells per 8-cm^2 well containing a glass coverslip and F-12 medium plus 10% FCS and grown for 62 h with a medium change at 48 h. Coverslips were prepared and viewed with differential-interference contrast (*a* and *c*) or fluorescence (*b* and *d*) microscopy as described in Materials and Methods. Bar,

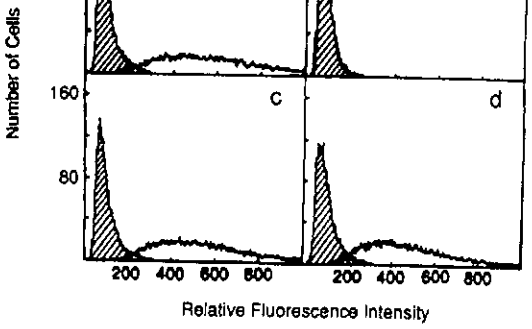

Figure 2. Flow cytometric analysis of Nile red–stained 25-RA (*a*); AC29 (*b*); and a revertant, 29CS1 (*c*), and transformant, 29T1 (*d*), of AC29. 2×10^5 cells were plated in 8-cm² wells and grown as described in Fig. 1. 29T1 cells were grown in the presence of 100 μg/ml G418. Cells were resuspended in buffer containing 100 ng/ml Nile red and analyzed by flow cytometry as described in Materials and Methods. Each histogram corresponds to 10^4 cells and the hatched histograms represent cells that had been plated and grown in medium containing 200 ng/ml 58-035.

When a different filter set is used (one that allows light >520 nm to be seen) the perinuclear droplets in 25-RA cells appeared yellow-gold and the diffuse signal in AC29 cells appeared orange-red. Greenspan and Fowler (22) have demonstrated that at low concentrations (100 ng/ml), Nile red emitted maximal fluorescence at 576 nm (yellow-gold) when it partitioned into hydrophobic environments, such as neutral lipid droplets. When Nile red interacted with phospholipids, maximal emission of fluorescence occurred at 628 nm (red). Apparently all the Nile red taken up by 25-RA cells is associated with the lipid droplets, so no diffuse staining due to other more polar lipids is seen.

It was previously shown that after Nile red staining, cholesterol ester–loaded, mouse peritoneal macrophages could be distinguished from unloaded macrophages by the cytofluorograph (23). As shown in Fig. 2, this was also true in CHO cells. A sevenfold difference was found in relative mean fluorescent intensities between Nile red–stained AC29 and 25-RA cells when analyzed for green fluorescence (mean ± SD for AC29 cells, 83.5 ± 34.4; and 25-RA cells, 578.8 ± 229.1). When grown in the presence of 58-035, the profile of AC29 cells was unaffected, but that of 25-RA was dramatically altered to become almost identical to AC29 (Fig. 2, *a* and *b*; *hatched curves*).

AC29 cells were mutagenized with *N*-nitroso-*N*-ethylurea and grown for 5–6 d to allow time for an altered phenotype to be expressed. The mutagenized cells were then stained with Nile red and sterilely sorted with the cytofluorograph as described in Materials and Methods. After two rounds of sorting, two putative revertants were isolated independently, 29CS1 and 29CS3. After Nile red staining, these cell lines had an identical fluorescent profile compared to 25-RA as analyzed by the cytofluorograph (Fig. 2 *c* and data not

tremely low (2–10%). Changes in the flow rate of the sorting (500–2,000 cells/s) or the time between staining and sorting (15–120 min) did not affect the survival rate (data not shown). To determine the cause of cell death, the experiment described in Table I was performed. Unstained or Nile red–stained cells were plated directly into dishes (200 cells/dish), or 200 of the 0.2% brightest cells were sorted via cytofluorography into dishes. For unsorted cells, the results indicated that Nile red had no effect on cell plating efficiency, which ranged from 25 to 41% for all cell types examined. There was a small reduction in cell survival in all cell types when unstained cells were passed through the flow cytometer (11–19%). When Nile red–stained cells were sorted, only the cell types with high Nile red fluorescence had survival rates comparable to unstained cells. The cells with low Nile red fluorescence (AC29 and AC29-G418^r, which are AC29 cells transfected with the plasmid pSV2-neo as described below) had a two- to fivefold lower survival rate than the other cell types. For our particular purpose, this was an unforeseen advantage, since AC29 cells were being selected against by their lower fluorescence and by a lower survival rate.

Approximately 7×10^6 mutagenized AC29 cells were sorted and ~300 cells (from six separate groups) survived. From these survivors, two independent revertants were isolated after an additional round of sorting. At the setting used for the first sort, 0.1% of all AC29 cells and ~40% of all 25-RA cells would be sorted. Since 29CS1 and 29CS3 cells have almost identical fluorescent profiles to 25-RA (Fig. 2 *c* and data not shown), there was an $\sim 4 \times 10^2$–fold enrichment of the revertants after the sort. In addition, there was probably a twofold difference in cell survival between the revertants and AC29 (Table I). Thus the frequency of isolation of revertants in this selection was $\sim 2/3 \times 10^2 \times 1/4 \times 10^2 \times 1/2 = 1/1.2 \times 10^5$.

Table I. Effect of the Fluorescent Stain Nile Red and the Cytofluorograph on Cell Survival

Cell type	Cell survival			
	Unsorted		Sorted	
	Unstained	Nile red	Unstained	Nile red
	%	%	%	%
25-RA	29.6 ± 3.3	30.6 ± 3.7	16.3 ± 2.0	23.6 ± 3.8
AC29	25.5 ± 2.2	28.6 ± 3.7	16.1 ± 2.2	7.1 ± 0.7
29CS1	28.3 ± 1.0	29.0 ± 2.5	19.3 ± 1.0	13.8 ± 3.3
AC29-G418^r	32.0 ± 0.5	32.1 ± 3.0	10.8 ± 3.0	3.3 ± 0.3
29T1	33.0 ± 4.7	41.0 ± 3.0	18.3 ± 2.7	16.6 ± 0.7

Monolayers were grown to confluency in F-12 medium plus 10% FCS (AC29-G418^r and 29T1 were grown in medium containing 100 μg/ml G418) and cells were resuspended in buffer with or without 100 ng/ml Nile red as described in Materials and Methods. Each sample was then subjected to the cytofluorograph and 200 of the 0.2% brightest cells were sorted into 100-mm dishes as described in Materials and Methods. In addition, the cell concentration of each unsorted sample was quantitated using a hemocytometer and 200 cells were plated directly into 100-mm dishes. After 10 d, the cell survival was determined by counting the number of colonies in each dish. The values are given as the mean of three dishes ± SD.

		Cholesterol ester
	nmol/mg protein	
25-RA	81.9 ± 7.7	234.0 ± 29.6
AC29	105.5 ± 21.6	3.8 ± 1.2
29CS1	72.1 ± 12.6	146.3 ± 13.7
29T1	77.8 ± 16.7	131.6 ± 15.0
25-RA (58-035)	86.8 ± 13.0	12.5 ± 2.9
AC29 (58-035)	81.6 ± 11.4	2.7 ± 5.1

5×10^5 cells were plated in 25-cm² flasks and grown in F-12 medium plus 10% FCS for 66 h with a medium change at 48 h and 64 h. 29T1 was grown in the presence of 100 μg/ml G418 throughout the experiment. The cells were harvested and analyzed for sterol content as described in Materials and Methods. Where indicated, 25-RA and AC29 were grown in medium containing 200 ng/ml 58-035. Duplicate aliquots were taken from duplicate dishes and the results are shown as the mean value ± SD.

Next, we transfected AC29 cells with exogenous DNA to determine if we could isolate transformants that had regained the ability to synthesize cholesterol ester. AC29 cells were cotransfected with high molecular weight DNA from human fibroblasts and pSV2-neo, a plasmid containing the gene conferring G418 resistance. The transfected cells were isolated by their acquired resistance to the toxic neomycin analogue, G418 (see reference 48). In several experiments, transfection frequencies ranging from 5×10^{-4} to 1.7×10^{-4} were obtained. It is known that cells transfected with selectable genes by the calcium phosphate precipitation technique also take up large amounts of the carrier DNA used (39), in our case human fibroblast genomic DNA. Of the 1.2×10^4 G418-resistant colonies obtained, one putative transformant, 29T1, was isolated (Fig. 2 *d*). The transformant had numerous Nile red–positive perinuclear particles similar to 25-RA and the revertants, which were not present when the cells were grown in the presence of the ACAT-specific inhibitor, 58-035 (data not shown).

Cholesterol Ester Metabolism in the Revertants and Transformant

As shown in Table II, analysis of the cholesterol ester mass of 25-RA, AC29, a revertant (29CS1), and the transformant (29T1) was consistent with the Nile red data, shown in Fig. 2. The majority of the cholesterol in 25-RA, 29CS1, and 29T1 cells was in the esterified form, the revertant and transformant always observed to have less cholesterol ester (~55–65%) than 25-RA cells. AC29 and cells grown in the ACAT inhibitor 58-035 had very little cholesterol ester. The values shown in Table II are almost twice as high as the values reported previously from this laboratory, although the results are qualitatively very similar (5). We believe this is due to differences in the preparation of the samples before the cholesterol analysis was performed (see Materials and Methods for details). The earlier method of preparing the samples, in which aliquots were taken from a NaOH-dissolved cell extract, has been found to be nonlinear in the amount of cholesterol detected with increasing volume of the aliquots (data not shown) which lead to an underestimation of the absolute values. The new method, in which the entire sample is

decrease in Nile red fluorescence and cholesterol ester when grown in 58-035 suggested that ACAT is active in these cells. To confirm this and to examine whether cholesterol ester synthesis is regulated normally in the isolated cell lines, the rate of cholesterol ester synthesis in response to LDL present in the growth medium was examined by [³H]oleate pulse (Fig. 3). LDL is known to activate ACAT in CHO cells (8, 17, 33). The rates of cholesterol ester synthesis in 25-RA, 29CS1, 29CS3, and 29T1 cells were all activated at least 50-fold by LDL while AC29 showed no response. In this experiment mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (1), was added to the cells shortly before and during the incubation with LDL to inhibit endogenous cholesterol biosynthesis, which also activates ACAT and thus partially masked the activation by LDL in 25-RA and its derived cell lines (data not shown). A small amount of mevalonate was added with the mevinolin, to permit the synthesis of nonsteroidal isoprenoids (45).

Table III shows the in vitro ACAT activities of the above mentioned CHO cells plus that of human fibroblasts. As seen

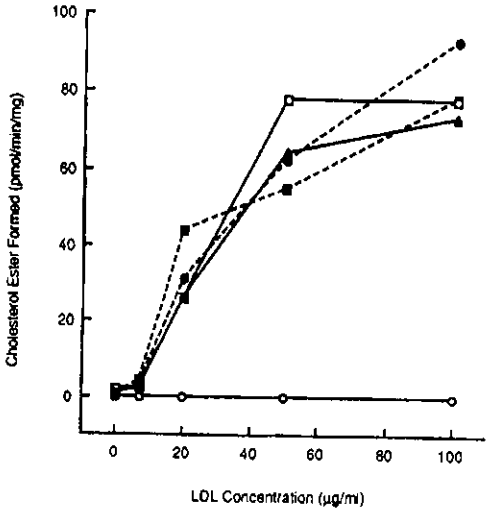

Figure 3. Activation of cholesterol ester synthesis by LDL in 25-RA (●; *dashed line*); AC29 (○; *solid line*); and two revertants, 29CS1 (□; *solid line*) and 29CS3 (■; *dashed line*), and a transformant, 29T1 (△; *solid line*), of AC29. 1.2×10^5 cells were plated in 25-cm² flasks and grown for 2 d in F-12 medium containing 10% FCS supplemented with $CaCl_2$ as described in Materials and Methods. After 2 d, the monolayers were washed three times with PBS and switched to F-12 medium supplemented with 10% delipidated FCS + 35 μM oleic acid and grown for an additional 36 h with a medium change 24 h after the switch. Then cells were changed to delipidated FCS medium containing 10 μM mevinolin and 230 μM mevalonate and grown for 6 h before a fresh medium containing increasing amounts of LDL was added. 29T1 cells were grown in the presence of 100 μg/ml G418 throughout this experiment. After 6 h of additional growth, the cells were pulsed with [³H]oleate and analyzed for cholesterol-[³H]oleate formed as described in Materials and Methods. Values are the average of duplicate flasks and ranged

Cell type	ACAT specific activity	
	Microsomal	Reconstituted
	pmol/min/mg	
25-RA	90.8	184.8
AC29	0.6	0.0
29CS1	68.8	91.1
29CS3	50.9	88.2
Human fibroblasts	6.3	30.1
29T1	69.5	67.8

For CHO cell lines, 3×10^5 cells were plated in 150-cm² flasks containing F-12 medium plus 10% FCS and grown for 66–70 h with a medium change at 48 h and 2 h before harvest. 29T1 was grown in the presence of 100 μg/ml G418 at all times. 7×10^3 human fibroblasts were plated in 150-cm² flasks containing MEM plus 10% FCS and grown for 7 d, with medium changes on day 4 and 6, and 2 h before harvest. Cells were harvested and assays were performed as described in Materials and Methods. The values are the means from duplicate assays and ranged within 10% of the mean.

previously, after reconstitution into cholesterol/phosphatidylcholine vesicles, the enzyme activity was elevated compared to the activity of the enzyme in the native microsomal membrane (4, 8, 17). In three separate experiments (Table III, Fig. 5, and data not shown), the reconstituted activities of 29CS1 and 29CS3 ranged from 39 to 51% and 42 to 48% of 25-RA–reconstituted ACAT activity, respectively. The ACAT activity of the putative transformant was tenfold higher than the activity found in human fibroblasts in the microsomal assay and approximately twice as high in the reconstitution assay.

Heat Stability of ACAT

As outlined in the previous section, the revertant and transformant cell lines isolated from AC29 have similar characteristics. Even though the frequency of obtaining the transformant was an order of magnitude higher than was found for the revertants isolated from mutagenized AC29 cells, the possibility existed that 29T1 was a G418-resistant clone which had reverted to an ACAT-positive phenotype during or after transfection.

While characterizing the ACAT activity of human fibroblasts, it was found that it was more stable at elevated temperatures than the enzyme activity of CHO cells. If 29T1 is an AC29 clone which has acquired the human ACAT gene, then its enzyme activity should have the heat stability characteristics similar to that found in human fibroblasts. This turned out to be the case. Fig. 4 shows the heat inactivation curves of microsomal enzyme activity at 45°C for the relevant cell lines. The inactivation curves, which do not follow simple first-order kinetics, revealed a distinct difference between the various cell types. The curves for the human fibroblasts and the transformant 29T1 were very similar, and demonstrated an enzyme activity substantially more heat stable than those of the other CHO cell lines. The curve for revertant 29CS1 was almost identical to the one for 25-RA, while the curve for the other revertant 29CS3 was much more heat labile.

The different heat inactivation curves between the cell lines examined in Fig. 4 could arise in part from differences

somes were performed. In this procedure the ACAT enzyme is solubilized from its native membrane by detergent and inserted into cholesterol-phospholipid vesicles of defined concentration (4). As shown in Fig. 5, all of the reconstituted enzyme activities were less stable than the microsomal activities, but the difference between the heat stability of the human and 25-RA enzyme was even more striking ($t_{½}$ for the human fibroblast enzyme was 5.5 min; for the 25-RA enzyme it was 1.3 min). The enzyme inactivation profile of 29T1 was almost identical to the one for human fibroblasts, while those for 25-RA, 29CS1, and 29CS3 were very similar.

When cells are transfected by the calcium phosphate coprecipitation technique used in this report, up to 2,000 kb of exogenous DNA are stably integrated into each transformant (39). Therefore, it is probable that many human genes have been taken up and expressed in the primary transformant, 29T1. To remove superfluous human DNA not involved in transforming AC29 to an ACAT-positive cell line, we isolated secondary transformants. This was accomplished by transfecting AC29 with pSV2-neo and whole genomic DNA isolated from 29T1 cells. Three secondary transformants, 29T2-4, 29T2-8, and 29T2-10, were isolated from 3.4×10^4 G418-resistant colonies. These cell lines had the

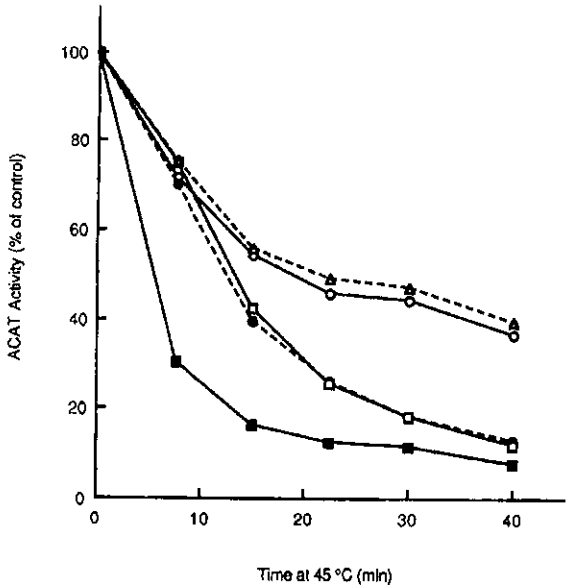

Figure 4. Heat inactivation of microsomal ACAT activity from cell extracts of 25-RA (●; *dashed line*); human fibroblasts (○; *solid line*); and two revertants, 29CS1 (□; *solid line*) and 29CS3 (■; *solid line*), and a transformant, 29T1 (△; *dashed line*), of AC29. CHO cells and human fibroblasts were grown and harvested as described in Table III. Cell extracts at 4°C were preincubated in a 20°C water bath for 3 min before incubation in a 45°C water bath for the indicated times. Samples were then placed on ice until assayed for enzyme activity as described in Materials and Methods. The control values for 25-RA, 29CS1, 29CS3, human fibroblasts, and 29T1 were 71.3, 51.0, 36.9, 5.9, and 68.5 pmoles/min per mg,

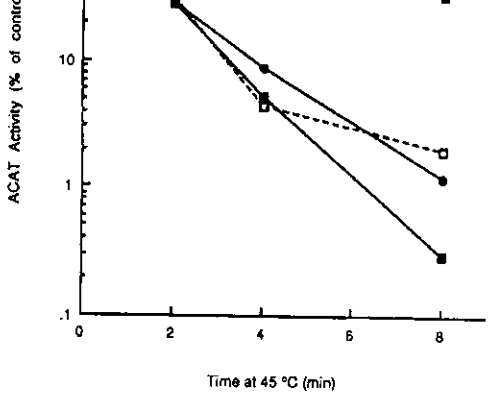

Figure 5. Heat inactivation of reconstituted ACAT activity from 25-RA (●; *solid line*); human fibroblasts (○; *solid line*); and two revertants, 29CS1 (□; *dashed line*) and 29CS3 (■; *solid line*), and a transformant, 29T1 (△; *dashed line*), of AC29. CHO cells and human fibroblasts were grown and harvested as described in Table III. Cell extracts were solubilized and diluted into cholesterol-phosphatidylcholine vesicles according to Cadigan and Chang (4). The reconstituted enzyme preparations were treated as described in Fig. 4 and assayed for activity. The control values for 25-RA, 29CS1, 29CS3, human fibroblasts, and 29T1 were 260.0, 101.2, 109.2, 52.0, and 93.9 pmoles/min per mg. Duplicates ranged within 10% of the mean.

perinuclear particles characteristic of 25-RA and the revertants and transformant isolated earlier as well as increased Nile red fluorescence and high ACAT activity. Fig. 6 demonstrated that the reconstituted enzyme activities of the secondary transformants had a rate of inactivation at elevated temperatures similar to the ones seen in the primary transformant and human fibroblasts, and distinct from the one in 25-RA cells.

Southern Analysis of Transformants Using a Labeled Human Repetitive Element

Alu repeats have been reported to be present in as many as 6–9 × 10^5 copies per haploid human genome (26, 42) and are found, on average, every few kilobases throughout the genome (52). To directly demonstrate that the isolated primary and secondary transformants have indeed stably integrated human DNA into their genomes, Southern analysis using a human repetitive element of the Alu family as the probe (44) was performed on restriction enzyme-digested, whole genomic DNA from the primary and secondary transformants. The results of one such experiment are shown in Fig. 7. The radiolabeled probe hybridized strongly to human DNA but not at all to AC29 DNA (Fig. 7, lanes *1* and *2*). Note that there is 2,000 times more AC29 DNA blotted onto the nylon filter than human DNA. The primary transformant, 29T1, was found to have a large amount of human sequences integrated into its genome. The secondary transformants, however, contained a relatively small amount of human sequences (see Fig. 7, lanes *4–9*). These results are common to all three secondary transformants in the Hind III/Eco RI double digest (Fig. 7, lanes *7–9*). Common bands of 23, 9.7, 6.8, and 2.2 kbp were also found in the Hind III digest (Fig. 7, lanes *4–6*). In addition, there were also bands common to all three transformants in Eco RI digests (data not shown). It is highly likely that these common fragments contain at least part of the gene which confers human ACAT activity to AC29 cells. The sum of the common bands in each digest added up to 26–42 kbp. The functional gene could be smaller than the sum of the common fragments, or larger due to significant portions of the gene that do not contain an Alu repeat. Further experiments will be needed to more exactly define the size of the functional human ACAT gene.

Discussion

This report describes a new selection procedure for the isolation of CHO cells that have regained their ability to synthesize cholesterol ester from a population of AC29 mutant cells which are deficient in ACAT activity. The selection procedure uses cytofluorography and Nile red, a fluorescent dye that partitions preferentially into neutral lipid droplets (22, 23). As shown in Fig. 2, the majority of the fluorescent signal is due to cholesterol ester synthesized intracellularly, since there is an approximate sevenfold reduction in fluorescence intensity when the cells were grown in the presence of the ACAT inhibitor 58-035. Although there is a small discrepancy between the flow cytometric data in Fig. 2 and the

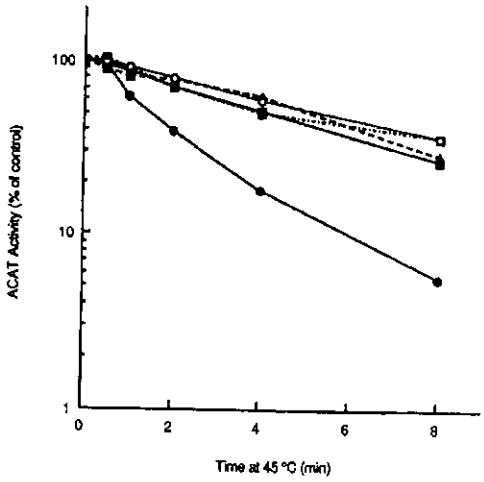

Figure 6. Heat inactivation of reconstituted ACAT activity from 25-RA (●; *solid line*); a primary transformant, 29T1 (△; *dashed line*); and three secondary transformants, 29T2-4 (□; *dashed/dotted line*), 29T2-8 (■; *solid line*), and 29T2-10 (○; *dotted line*), of AC29. CHO cells were grown and harvested as described in Table III. Extracts were reconstituted according to the procedure of Cadigan and Chang (4). The samples were treated as described in Fig. 4 and assayed for enzyme activity. The control activities for 25-RA, 29T1, 29T2-4, 29T2-8, and 29T2-10 were 137.2, 41.7, 39.6, 45.7, and 62.6 pmoles/min per mg. Duplicates ranged within 7% of the mean.

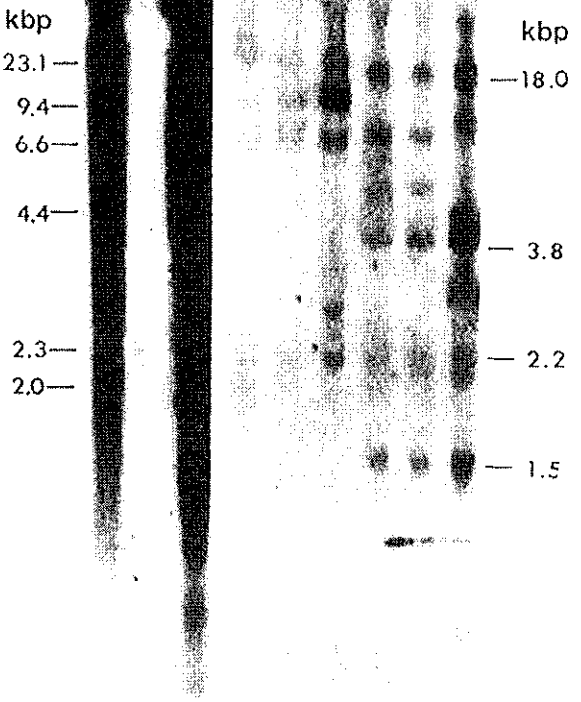

Figure 7. Southern analysis of enzyme-restricted, whole-genomic DNA from AC29, human fibroblasts, and the transformants probed with a radiolabeled human repetitive element. Genomic DNA of human fibroblasts (lane *1*), AC29 (lane *2*), 29T1 (lane *3*), 29T2-4 (lanes *4* and *7*), 29T2-8 (lanes *5* and *8*), and 29T2-10 (lanes *6* and *9*) were digested with Hind III (lanes *1–6*) or Hind III and Eco RI (lanes *7–9*), run on a 0.8% agarose gel, transferred to a nylon filter, and probed with radiolabeled Alu repetitive element as described in Materials and Methods. 20 μg of genomic DNA was used for each sample except for human fibroblasts (10 ng) and 29T1 (10 μg). A Hind III digest of lambda DNA served as molecular weight markers and are indicated on the left in kilobase pairs and the Eco RI/Hind III restriction fragments common to all the secondary transformants are indicated on the right in kilobase pairs.

chemical determination of cholesterol ester content in Table II (29CS1 and 29T1 have ~60% of the cellular cholesterol ester as 25-RA cells, while their Nile red fluorescent profiles are almost the same), Nile red can be viewed as an indirect assay for cholesterol ester in the CHO cell lines examined.

The survival of Nile red–stained AC29 cells after sorting through the cytofluorograph was very low. The data in Table I suggested that the Nile red staining plus cell sorting preferentially killed cholesterol ester–deficient cells, while cholesterol ester–rich cells are relatively unaffected. Although the reason for this specific killing is not known for certain, it may be that after excitation by the laser beam in the cytofluorograph, the Nile red compound becomes cytotoxic. In cells with large amounts of cholesterol ester, the toxic compound is trapped in the lipid droplets, thereby enabling the cells to survive. Since this is the first report using Nile red for sorting were enriched. However, it may prove difficult to use Nile red in selections where cells are selected for lower Nile red fluorescence.

The two revertants, 29CS1 and 29CS3, isolated from mutagenized populations of AC29 cells, have highly regulated cholesterol ester synthesis in intact cells and relatively high ACAT activity in vitro (Fig. 3 and Table III). The reconstituted enzyme activities of the revertants are between 39 and 51% of that found in 25-RA cell extracts (Table III, Fig. 5, and data not shown). This reconstitution assay is entirely dependent on the exogenous cholesterol present in the vesicles, which was at saturating amounts in our experiments, thus eliminating any differences in ACAT activities due to differences in the cholesterol composition of the microsomal membranes (4). Therefore, the revertants appear to have approximately half the enzyme content of 25-RA cells. The frequency of obtaining the revertants from mutagenized AC29 cells was calculated to be 8×10^{-6}, a value consistent with other reports of reversion of single genes (9, 13, 30, 38, 54). The low frequency of isolation of the ACAT mutants suggested that more than one gene needed to be inactivated to produce the ACAT mutant phenotype (5). We now propose, based on the biochemical data and the frequency of isolation of the revertants, that there are two active genes in the parental cell line 25-RA and that one of the two genes has been reactivated in the revertants.

A transformant was isolated after AC29 cells were transfected with whole genomic DNA obtained from human fibroblasts. This transformant, termed 29T1, appears to be very similar to the revertants with respect to cholesterol ester metabolism (Fig. 3 and Tables II and III). However, the heat inactivation curves shown in Figs. 4 and 5 clearly demonstrated that the ACAT activity of 29T1 has heat stability properties identical to that of the human fibroblast ACAT activity and distinct from the other CHO cells. AC29 could contain a defect in a structural gene encoding ACAT or in a gene needed for enzyme production. The heat inactivation data strongly favors the first possibility. If AC29 is a production mutant and transfection with human DNA corrected the mutation, the transformant's enzyme activity should have biochemical properties similar to that found in CHO cells, not human fibroblasts.

Three secondary transformants were isolated independently by transfecting AC29 with genomic DNA from 29T1 and were shown to possess human-like enzyme activity (Fig. 6). The finding that all three secondary transformants had heat stability properties similar to the primary transformant suggested that they have acquired the heat-stable (i.e., human) gene from the 29T1 genomic DNA used in the transfection. With the transfection protocol used, a very small amount of human DNA should remain in the secondary transformants (28, 35, 37, 46). This was confirmed by Southern analysis using a human repetitive element as the probe. Restriction fragments common to all three secondary transformants allowed a tentative approximation of the size of the structural gene for the human ACAT enzyme. We cannot rule out the possibility of more than one structural gene for the enzyme, but at present there is no genetic or biochemical evi-

activity is only twice as high. This suggests that the enzyme has more cholesterol available to it in the CHO cell microsomal membrane than in the fibroblasts, not surprising since 29T1 is a 25-RA-derived cell line and has an elevated rate of cholesterol biosynthesis and increased number of LDL receptors compared to wild-type CHO cells (data not shown). The remaining twofold difference in enzyme activity could be explained by an increased level of expression, or a CHO cell-specific posttranslational modification of the human ACAT enzyme in the transformants.

In conclusion, the results reported in this paper strongly suggest that the molecular lesion in the ACAT-deficient mutants isolated previously in this laboratory (5) resides in the structural gene for the enzyme, which either prevents expression or leads to the production of an inactive enzyme. There are normally two active ACAT genes in CHO cells, but the reversion or transfection of one ACAT gene is sufficient to restore an ACAT-positive phenotype. The secondary transformants isolated possess one human gene encoding the ACAT enzyme and all three contain common restriction enzyme fragments which hybridize to a cloned human-specific repetitive element. These common fragments probably form part or all of the human ACAT gene. It should now be possible to isolate the gene from a secondary transformant genomic library, as has been accomplished for other human genes (28, 35, 46). The isolation of a functional ACAT gene will be the first step towards the preparation of the molecular tools necessary for probing the molecular structure and regulation of this enzyme.

The authors would like to thank Dr. Paul Guyre (Department of Physiology, Dartmouth Medical School) for advice concerning cell sorting and Gary Ward for excellent operation of the cytofluorograph. We also thank Drs. Robert Jackson and Stanley Froehner (Department of Biochemistry, Dartmouth Medical School) for the use of their Zeiss universal microscope and Diane Spillane and Shan-ping Shi for careful reading of this manuscript.

The cytofluorograph used in this study was the generous gift of the Fannie E. Rippel Foundation, and is partially supported by the core grant of the Norris Cotton Cancer Center (Hanover, NH) (CA 23108). This work was supported by National Institutes of Health Grant HL 36709.

Received for publication 28 October 1988 and in revised form 31 January 1989.

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Morphology: lymphoblast

Source: **Cell Type: hybridoma:** B lymphocyte;

Cellular Products: immunoglobulin; monoclonal antibody; against bovine low density lipoprotein (LDL) receptor

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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

Related Links

- NCBI Entrez Search
- Cell Micrograph
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Login Required

- Product Information Sheet

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

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 (CO_2), 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-12006
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

References:

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Return to Top

Back to my Search

Product Description

Print this Page

Cell Biology

ATCC® Number: HB-8065™ Order this Item

Price: $272.00

Designations: Hep G2

Depositors: Wistar Institute

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

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]

Related Links

NCBI Entrez Search

Cell Micrograph

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Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

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 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
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

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 medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CRL-10741
derivative:ATCC CRL-11997
purified DNA:ATCC HB-8065D

References:

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informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate

All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

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Catalog ID: **GM02036**

Product (Source): CELL CULTURE

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

Bio Safety level 2

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  - Online Ordering
  - Placing First Online Order
  - Assurance Form Signatory
  - Frozen Shipments Policy
- FAQs
  - Lymphoblast Cultures
  - Fibroblast Cultures
  - Fetal Bovine Serum
  - Searching
  - Cell Types
  - Culture Medium
  - ISCN Nomenclature
  - Searching Mutations
  - Quick Search
  - Safety Guidelines
  - Quality Control
  - Passage vs PDL
- Miscellaneous
  - Feedback
  - Links
  - Contact Us
  - Cell Collections

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Unspecified
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age At Sampling 11 YR
Sex Female
Race Caucasian
Family 203 View Pedigree
Family Member 2
Relation to Proband sister
Clinically Affected No
Confirmation Karyotypic analysis and Case history
ISCN 46,XX
Remarks Skin biopsy; sister of GM02037C; 46,XX; 4% of cells show random chromosome loss

Catalog ID GM02036
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 13

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

Phenotypic Data

Remark Skin biopsy; sister of GM02037C; 46,XX; 4% of cells show random chromosome loss

Publications

Merla G, Howald C, Henrichsen CN, Lyle R, Wyss C, Zabot MT, Antonarakis SE, Reymond A, Submicroscopic deletion in patients with williams-beuren syndrome influences expression levels of the nonhemizygous flanking genes American journal of human genetics79:332-41 2006
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Reed GP, Rainford DJ, Reed GP, Brannon PM, Spielberg SP, Schulman JD, Glutathione metabolism in normal and cystinotic fibroblasts. Exp Cell Res172:158-67 1987
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Guernsey DL, Koebbe M, Thomas JE, Myerly TK, Zmolek D, An altered response in the induction of cell membrane (Na + K) ATPase by thyroid hormone is characteristic of senescence in cultured human fibroblasts. Mech Ageing Dev33:283-93 1986
PubMed ID: 3012221

External Links

dbSNP dbSNP ID: 14247

Images

View pedigree

Culture Protocols

Passage Frozen 13
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 10% fetal bovine serum Not inactivated
Substrate None specified
Subcultivation Method trypsin-EDTA

Catalog ID: **GM03123**

Product (Source): CELL CULTURE

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  - Online Ordering
  - Placing First Online Order
  - Assurance Form Signatory
  - Frozen Shipments Policy
- FAQs
  - Lymphoblast Cultures
  - Fibroblast Cultures
  - Fetal Bovine Serum
  - Searching
  - Cell Types
  - Culture Medium
  - ISCN Nomenclature
  - Searching Mutations
  - Quick Search
  - Safety Guidelines
  - Quality Control
  - Passage vs PDL
- Miscellaneous
  - Feedback
  - Links
  - Contact Us
  - Cell Collections

Biosafety level 2

- Overview
- Characterizations
- Phenotypic Data
- Publications
- External Links
- Images
- Culture 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 At Sampling 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 isoluecine 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
PDL at Freeze 5.74
Passage Frozen 9

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

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 isoluecine at codon 1061 [Ile1061Thr (I1061T)] in a transmembrane domain.

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PubMed ID: 18375996

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PubMed ID: 15347664

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PubMed ID: 15256387

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Maziere JC, Maziere C, Mora L, Routier JD, Polonovski J, In situ degradation of sphingomyelin by cultured normal fibroblasts and fibroblasts from patients with Niemann-Pick disease type A and C. Biochem Biophys Res Commun108:1101-6 1982
PubMed ID: 7181884

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

Percent O2 AMBIENT

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

Product Description

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Cell Biology

ATCC® Number: CRL-2545™ Order this Item

Price: $399.00

Designations: IgG-1D2

Depositors: JL Goldstein, YK Ho

Isotype: IgG1; kappa light chain

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension

Organism: Mus musculus (B cell); Mus musculus (myeloma) (mouse (B cell); mouse (myeloma))

Morphology: lymphoblast

Source: **Cell Type: hybridoma:** B lymphocyte;

Cellular Products: immunoglobulin; monoclonal antibody; against human sterol regulatory element binding protein 2 (SREBP 2, SREBP-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.

Comments: Animals were immunized against the GST-human SREBP-2 fusion protein (amino acids 48 to 403).
Spleen cells were fused with Sp2/0-Ag14 myeloma cells.
The antibody recognizes both the precursor and mature forms of SREBP-2 in cultured human cells, and can be used in immunoblotting and ELISA assays to study SREBP-2 in cultured human cells grown under different conditions.

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

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Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2 X 10 exp5 viable cells/ml. Maintain cell density between 1 X 10 exp5 and 1 X 10 exp6 viable cells/ml.

Preservation: culture medium 95%; DMSO, 5%

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

References: 56311: Janowski BA, et al. The hypocholesterolemic agent LY295427 reverses suppression of sterol regulatory element-binding protein processing mediated by oxysterols. J. Biol. Chem. 48: 45408-45416, 2001. PubMed: 11577112

Return to Top

Back to my Search

Product Description

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Cell Biology

ATCC® Number: TIB-67™ Order this Item

Price: $256.00

Designations: J774A.1

Depositors: P Ralph

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: macrophage

Source:
Tissue: ascites
Strain: BALB/cN
Disease: reticulum cell sarcoma
Cell Type: monocyte/macrophage macrophage;

Cellular Products:
interleukin 1 beta
lysozyme [1080]

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:
La Jolla California, United States
Isolation date: 1968

Applications:
Biological response [92560]
transfection host (Roche FuGENE® Transfection Reagents)

Receptors:
complement (C3), expressed [1135]

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phagocytosis. Interleukin 1 beta (Il1b) is synthesized continuously by this 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%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:

Protocol: Subcultures are prepared by scraping.
For a 75 cm2 flask, remove all but 10 ml of the culture medium. (adjust volume accordingly for different culture vessels) Dislodge cells from the flask substrate with a cell scraper, aspirate and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Replace or add medium two or three times weekly

Preservation:

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

Doubling Time:

17 hours

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
purified RNA:ATCC TIB-67R

References:

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1135: Ralph P, Nakoinz I. Antibody-dependent killing of erythrocyte and tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031
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32697: Knowlton KU, et al. A mutation in the puff region of VP2 attenuates the myocarditic phenotype of an infectious cDNA of the woodruff variant of coxsackievirus B3. J. Virol. 70: 7811-7818, 1996. PubMed: 8892902
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92560: Standard Practice for Testing for Biological Responses to Particles in Vitro. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 1903-98R03.

Return to Top

Back to my Search

Product Description

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Cell Biology

ATCC® Number: CRL-1601™ Order this Item

Price: $331.00

Designations: McA-RH7777

Depositors: JE Becker

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: loosely adherent

Organism: Rattus norvegicus (rat)

Morphology: epithelial

Source:
Organ: liver
Strain: Buffalo
Disease: hepatoma; Morris hepatoma 7777

Cellular Products: alpha-fetoprotein (AFP, alpha fetoprotein)

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

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Receptors: glucocorticoid

Gender: female

Comments: Addition of glucocorticoids (dexamethasone) to the medium accelerates cell proliferation and reduces alpha fetoprotein production.

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

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If any cells are attached, tap flask gently or if necessary add 2.0 to 3.0 ml of 0.25% Trypsin-0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed . 3. Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting. 4.To remove trypsin-EDTA solution, transfer cell suspension to the centrifuge tube with the medium and cells from step #1 and spin at approximately 125 xg for 5 to10 minutes.5. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. 6. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 weekly is recommended

Medium Renewal: Add medium every 2 to 3 days. do not discard floating cells.

Preservation:

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

Related Products:

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

References:

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Return to Top

Back to my Search

Product Description

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Cell Biology

ATCC® Number: HTB-22™ [Order this Item]

Price: $272.00

Designations: MCF7

Depositors: CM McGrath

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

Cellular Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

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: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

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Product Information Sheet

D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

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

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

Age: 69 years adult

Gender: female

Ethnicity: Caucasian

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

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% .
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

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

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Doubling Time: 29 hrs

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-22D
purified RNA:ATCC HTB-22R
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7 cells. Int. J. Cancer 55: 453-458, 1993. PubMed: 8375929

23046: Brandes LJ, Hermonat MW. Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol. Cancer Res. 43: 2831-2835, 1983. PubMed: 6850594

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25065: Bellet D, et al. Malignant transformation of nontrophoblastic cells is associated with the expression of chorionic gonadotropin beta genes normally transcribed in trophoblastic cells. Cancer Res. 57: 516-523, 1997. PubMed: 9012484

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32285: van Dijk MA, et al. A functional assay in yeas for the human estrogen receptor displays wild-type and variant estrogen receptor messenger RNAs present in breast carcinoma. Cancer Res. 57: 3478-3485, 1997. PubMed: 9270016

32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029

32344: Umekita Y, et al. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. Proc. Natl. Acad. Sci. USA 93: 11802-11807, 1996. PubMed: 8876218

32467: Zamora-Leon SP, et al. Expression of the fructose transporter GLUT5 in human breast cancer. Proc. Natl. Acad. Sci. USA 93: 1847-1852, 1996. PubMed: 8700847

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38764: Bacus SS, et al. Differentiation of cultured human breast cancer cells (AU-565 and MCF-7) associated with loss of cell surface HER-2/neu antigen. Mol. Carcinog. 3: 350-362, 1990. PubMed: 1980588

Return to Top

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:	**HTB-26™** Order this Item	**Price:**	**$256.00**
Designations:	MDA-MB-231		
Depositors:	R Cailleau		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		

Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed
Tumorigenic:	Yes
DNA Profile (STR):	

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

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

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

Age: 51 years adult

Gender: female

Ethnicity: Caucasian

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

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 100%
Temperature: 37.0°C

Subculturing:

Protocol:

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

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

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

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

331, 1976.
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Return to Top

Back to my Search

Product Description

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Cell Biology

ATCC® Number: TIB-61™ Order this Item

Price: $438.00

Designations: PU5-1.8 (PU5-1R)

Depositors: P Ralph

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension (some adherent cells)

Organism: *Mus musculus* (mouse)

Morphology:

Source: **Disease:** lymphoid tumor
Strain: BALB/c

Cellular Products: lysozyme; granulocyte colony stimulating activity (CSA) inducible by LPS

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.

Receptors: complement (C3)

Comments: PU5-1.8 cells phagocytose latex beads and zymosan.
The cells are capable of antibody dependent lysis of both sheep erythrocytes and tumor cells.
The line is sensitive to growth inhibition by LPS and PPD.
Tested and found negative for ectromelia virus (mousepox).

Propagation: **ATCC complete growth medium:** Dulbecco's modified Eagle's medium, 90%; horse serum, 10%

Subculturing: **Medium Renewal:** Every 2 to 3 days
Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 cells/ml and maintain between 1 X 10 exp5

Related Links

NCBI Entrez Search

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

tumor targets by macrophage-related cell lines: enhancement by PPD and LPS. J. Immunol. 119: 950-954, 1977. PubMed: 894031
1136: Ralph P, Nakoinz I. Direct toxic effects of immunopotentiators on monocytic myelomonocytic, and histiocytic or macrophage tumor cells in culture. Cancer Res. 37: 546-550. 1977. PubMed: 318922
1137: Ralph P, Nakoinz I. Lipopolysaccharides inhibit lymphosarcoma cells of bone marrow origin. Nature 249: 49-51, 1974. PubMed: 4208429

Return to Top

Back to my Search

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.

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.

Print this Page

Cell Biology

ATCC® Number: TIB-202™ [Order this Item]

Price: $272.00

Designations: THP-1

Depositors: S Tsuchiya

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension

Organism: *Homo sapiens* (human)

Morphology: monocyte

Source:
Organ: peripheral blood
Disease: acute monocytic leukemia
Cell Type: monocyte;

Cellular Products: lysozyme [58053]

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: complement (C3), expressed [58053]
Fc, expressed

Antigen Expression: HLA A2, A9, B5, DRw1, DRw2 [58053]

DNA Profile (STR):

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

D16S539: 11,12
D5S818: 11,12
D7S820: 10
THO1: 8,9.3
TPOX: 8,11
vWA: 16

Age: 1 year infant

Gender: male

Comments: The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053]
Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [22193]

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 2-4 X 10(5) viable cells/ml. Subcultutre when cell concentration reaches 8X105) cells/ml.Do not allow the cell concentration to exceed 1 X 10(6) cells/ml.
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

Doubling Time: approximately 26 hrs

Related Products: purified RNA:ATCC TIB-202R
purified DNA:ATCC TIB-202D
Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2001
Recommended serum: ATCC 30-2020
Cell culture tested DMSO: ATCC 4-X

References:

by murine monoclonal antibodies. J. Immunol. 131: 1882-1888, 1983. PubMed: 6619543
32286: Cuthbert JA, Lipsky PE. Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. Cancer Res. 57: 3498-3504, 1997. PubMed: 9270019
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
32395: Clark RA, et al. Tenascin supports lymphocyte rolling. J. Cell Biol. 137: 755-765, 1997. PubMed: 9151679
32466: Hambleton J, et al. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad Sci. USA 93: 2774-2778, 1996. PubMed: 8610116
33031: Hsu HY, et al. Inhibition of macrophage scavenger receptor activity by tumor necrosis factor-alpha is transcriptionally and post-transcriptionally regulated. J. Biol. Chem. 271: 7767-7773, 1996. PubMed: 8631819
33088: Lucas M, Mazzone T. Cell surface proteoglycans modulate net synthesis and secretion of macrophage apolipoprotein E. J. Biol. Chem. 271: 13454-13460, 1996. PubMed: 8662812
33134: Sando GN, et al. Induction of ceramide glucosyltransferase activity in cultured human keratinocytes. J. Biol. Chem. 271: 22044-22051, 1996. PubMed: 8703011
33141: Ollivier V, et al. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. J. Biol. Chem. 271: 20828-20835, 1996. PubMed: 8702838
58053: Tsuchiya S, et al. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int. J. Cancer 26: 171-176, 1980. PubMed: 6970727

Return to Top

Back to my Search

COMPANY/UNDERTAKING

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

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

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

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

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

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

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. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
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

Exposure limits

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

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	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

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

For research use only

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 present unknown hazards and should be used with caution. Since the Company 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, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

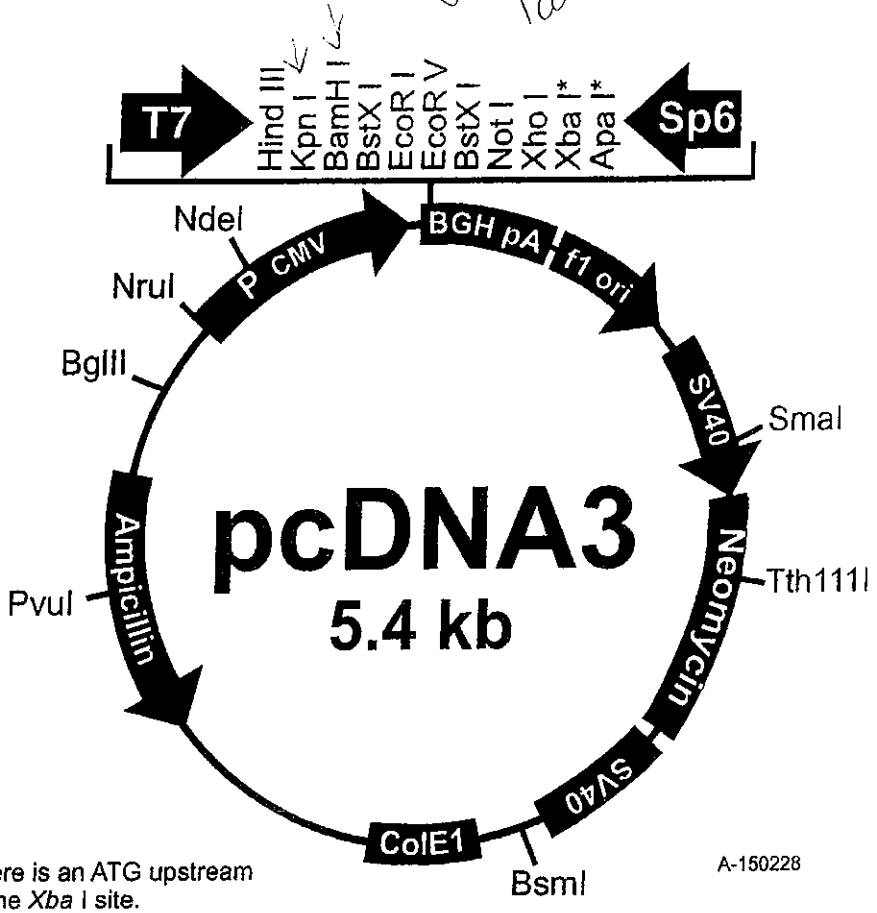
End of Safety Data Sheet

Comments for pcDNA3:
5446 nucleotides

CMV promoter: bases 209-863
T7 promoter: bases 864-882
Polylinker: bases 889-994
Sp6 promoter: bases 999-1016
BGH poly A: bases 1018-1249
SV40 promoter: bases 1790-2115
SV40 origin of replication: bases 1984-2069
Neomycin ORF: bases 2151-2945
SV40 poly A: bases 3000-3372
ColE1 origin: bases 3632-4305
Ampicillin ORF: bases 4450-5310

This vector that ERd came to lab in from Gabi's lab.

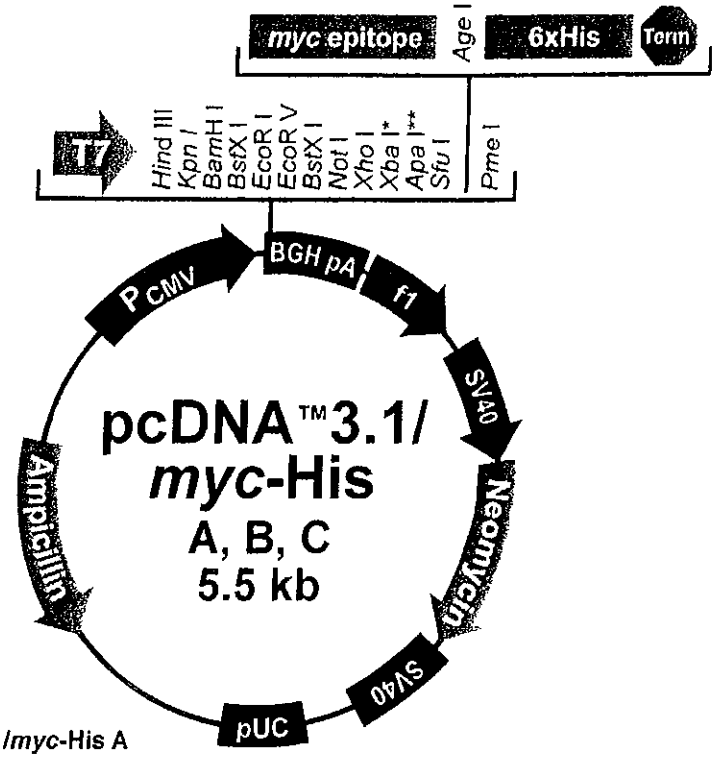
Invitrogen
life technologies



* There is an ATG upstream of the *Xba* I site.

A-150228

The sequence of pcDNA3 has been compiled from information in sequence databases, published sequences, and other sources. This vector has not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department.

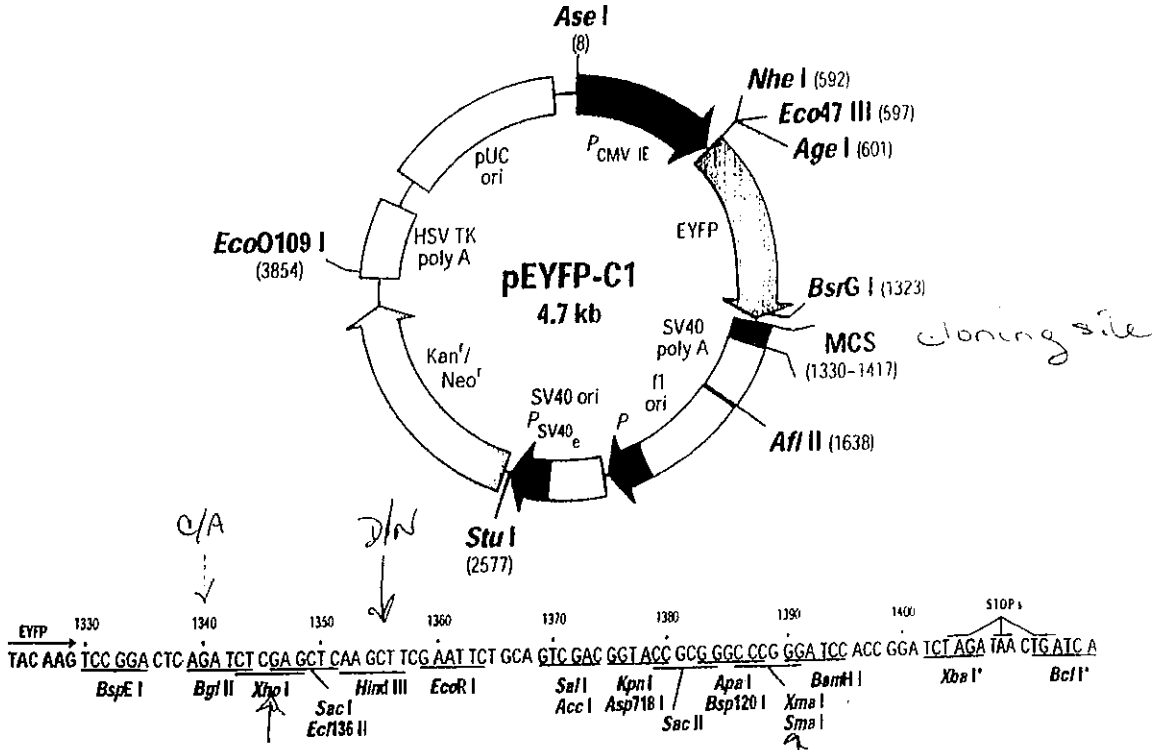


Comments for pcDNA™3.1/*myc*-His A
5493 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
myc epitope: bases 997-1026
Polyhistidine tag: bases 1042-1059
BGH reverse priming site: bases 1082-1099
BGH polyadenylation signal: bases 1081-1295
f1 origin of replication: bases 1358-1771
SV40 promoter and origin: bases 1836-2160
Neomycin resistance gene: bases 2196-2990
SV40 polyadenylation signal: bases 3166-3296
pUC origin: bases 3679-4352
Ampicillin resistance gene: bases 4497-5357 (complementary strand)

* There is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.



Restriction map and multiple cloning site (MCS) of pEYFP-C1. All restriction sites are shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-C1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513-nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-C1 is between the EYFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening in-frame stop codons. EYFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection

II. Product Components

Product	Size	Cat.#
pRL-TK Vector	20µg	E2241

All pRL Vectors are supplied in TE buffer (pH 7.4) and are provided with a glycerol stock of bacterial strain JM109. The JM109 cells do not contain vector and are not competent cells.

Storage Conditions: Store vector DNA at –20°C and the glycerol stock of JM109 cells at –70°C.

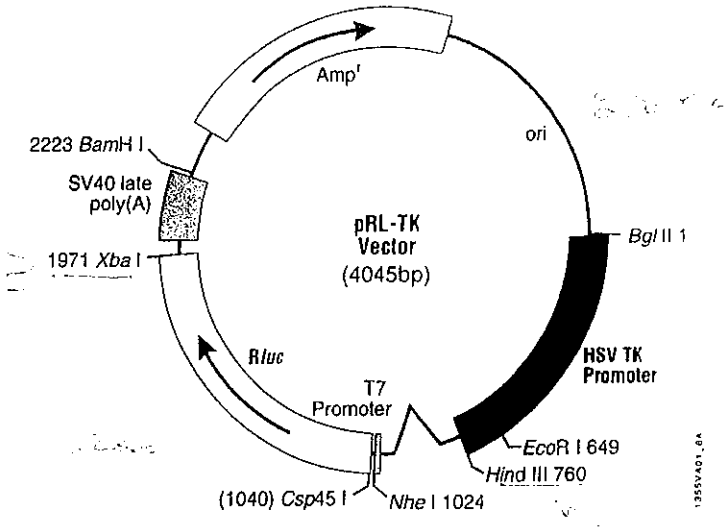


Figure 1. The pRL-TK Vector circle map and sequence reference points.

Sequence reference points:	
HSV-TK promoter	7–759
Chimeric intron	826–962
T7 RNA polymerase promoter (–17 to +2)	1006–1024
T7 RNA polymerase transcription initiation site	1023
R*luc* reporter gene	1034–1969
SV40 late polyadenylation signal	2011–2212
β-lactamase (Amp^r) coding region	2359–3219

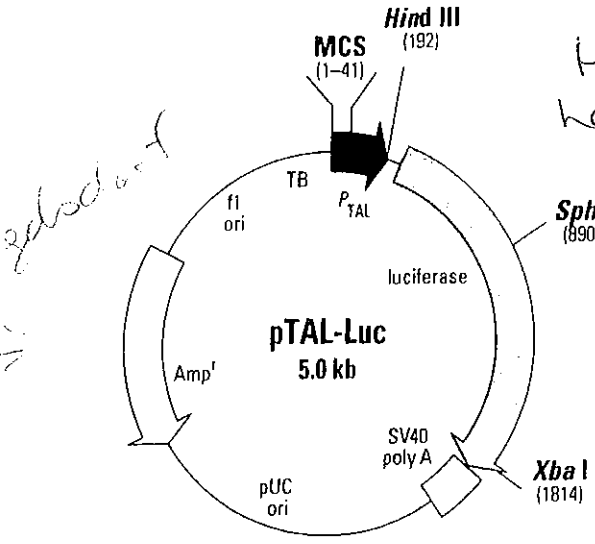
In addition:

- ⌒ indicates the position of the intron.
- R*luc* is the cDNA encoding the *Renilla* luciferase enzyme.
- Amp^r indicates the gene encoding ampicillin resistance in *E. coli*.
- ori is the origin of replication in *E. coli*.
- The arrows within the R*luc* and Amp^r genes indicate the direction of transcription. Restriction sites shown in parentheses are not unique sites.

pTAL-Luc Vector Information

GenBank Accession #: Submission in progress.

Catalog #6252-1



Restriction Map and Multiple Cloning Site (MCS) of pTAL-Luc. All sites shown are unique.

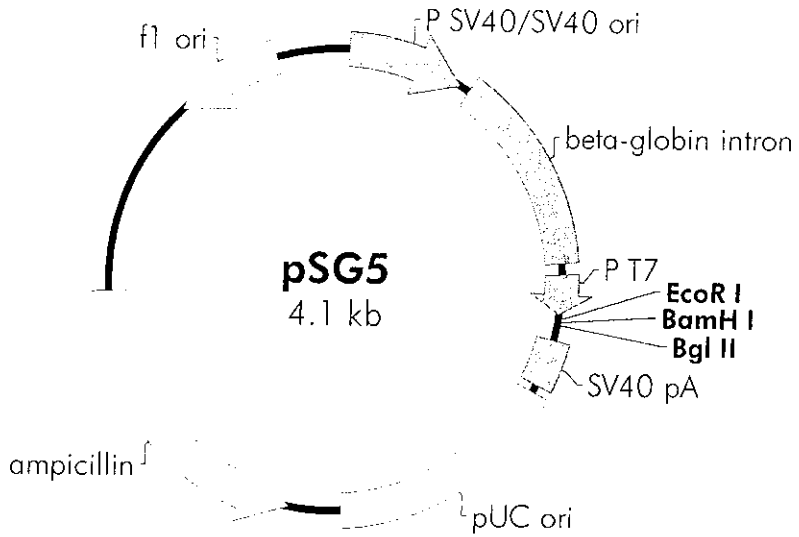
Description:

pTAL-Luc is designed for analyzing enhancer sequences by assaying for expression of the firefly luciferase (*luc*) gene from *Photinus pyralis* (1). This vector contains a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. Putative enhancers can be inserted into one of the MCSs. The luciferase coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the *luc* transcript in eukaryotic cells. Located upstream of the MCS is a synthetic transcription blocker (TB), which is composed of adjacent polyadenylation and transcription pause sites for reducing background transcription (2). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E. coli*.

Use:

pTAL-Luc is ideal for use as a negative control or for studying putative enhancers that are inserted upstream of the luciferase reporter gene. Luciferase is a highly sensitive enzymatic reporter that can be assayed by any standard luciferase-detection method, providing quantitative data on induction levels. The pTAL-Luc Vector can be transfected into mammalian cells by any standard method. For selecting stable clones, cotransfect with a vector containing an antibiotic resistance gene, such as neomycin, hygromycin or puromycin.

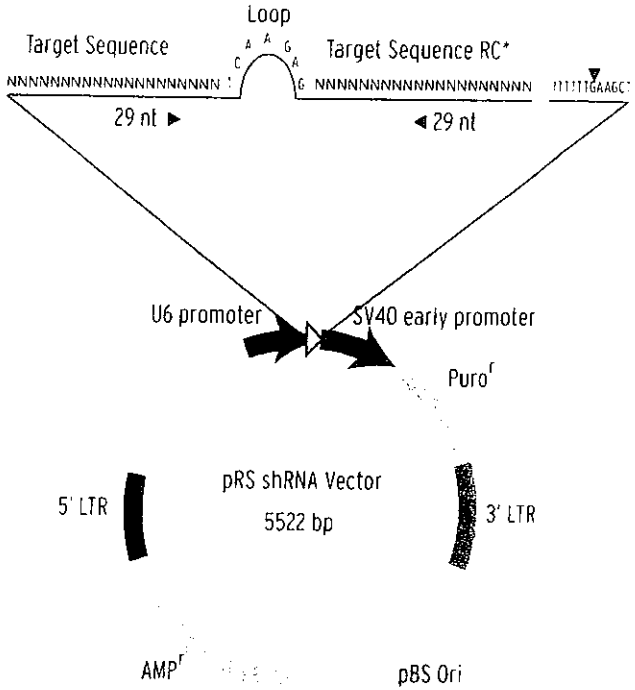
The pSG5 Vector



Feature	Nucleotide Position
SV40 promoter and SV40 origin of replication	28–366
β-globin intron	395–967
T7 promoter	1022–1040
EcoR I	1043
BamH I	1049
Bgl II	1055
SV40 polyA signal	1069–1202
pUC origin of replication	1342–2009
ampicillin resistance (*bla*) ORF	2160–3017
f1 origin of ss-DNA replication	3587–3893

Figure 1 Circular map and features of the pSG5 vector. The complete sequence and list of restriction sites is available at *www.stratagene.com*.

pRS vector map



*RC: reverse component

Material Safety Data Sheet

Version 4.0
Revision Date 03/13/2010
Print Date 05/03/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Pertussis toxin, from *Bordetella pertussis*

Product Number : P7208
Brand : Sigma

Company : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Telephone : +19058299500
Fax : +19058299292
Emergency Phone # : 800-424-9300

Toxin Info

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Pancreas.

WHMIS Classification

D1A Very Toxic Material Causing Immediate and Serious Toxic Effects
D1B

Highly toxic by inhalation
Toxic by ingestion
Toxic by skin absorption

GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)

H302	Harmful if swallowed.
H311	Toxic in contact with skin.
H330	Fatal if inhaled.

Precautionary statement(s)

P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing.
P284	Wear respiratory protection.
P310	Immediately call a POISON CENTER or doctor/physician.

HMIS Classification

Health hazard: 4
Chronic Health Hazard: *
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be fatal if inhaled. May cause respiratory tract irritation.
Skin Toxic if absorbed through skin. May cause skin irritation.

IAP
Pertussigen
Histamine-sensitizing factor

CAS-No.	EC-No.	Index-No.	Concentration
Pertussis toxin from Bordetella pertussis			
70323-44-3	-	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Face shield and safety glasses

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	powder, lyophilized

Safety data

pH	no data available
Melting point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

11. TOXICOLOGICAL INFORMATION

Acute toxicity
LD50 Intravenous - rat - 0.114 mg/kg
Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste):Eye:Lacrimation. Behavioral:Change in motor

no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure (GHS)
no data available

Specific target organ toxicity - repeated exposure (GHS)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be fatal if inhaled. May cause respiratory tract irritation.
Ingestion	Toxic if swallowed.
Skin	Toxic if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
Potentially neurotoxic.

Additional Information
RTECS: XW5883750

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging

Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 3462 Class: 6.1 Packing group: I EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Pertussis toxin from Bordetella pertussis)
Marine pollutant: No

IATA
UN-Number: 3462 Class: 6.1 Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Pertussis toxin from Bordetella pertussis)

15. REGULATORY INFORMATION

DSL Status
This product contains the following components that are not on the Canadian DSL nor NDSL lists.

	CAS-No.
Pertussis toxin from Bordetella pertussis	70323-44-3

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and	Highly toxic by inhalation
D1B	Serious Toxic Effects	Toxic by ingestion
		Toxic by skin absorption

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Pertussis
Proposed Use Dose:	1 µg
Proposed Storage Dose:	1 µg
LD_{50} (species):	18 µg

Calculation:

18 µg/kg x 70 kg/person

Dose per person based on LD_{50} in µg = 1260

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 126

Comments/Recommendations: OK