

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOHAZARDOUS AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: March 27, 2009  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

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

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

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto: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/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR  
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Location of experimental work to be carried out: Building(s) SDMRI Room(s) 237, 238

\*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CANADIAN CANCER SOCIETY  
GRANT TITLE(S): MOLECULAR ANALYSIS OF NOVEL MECHANISMS  
REGULATING MAMMALIAN GENOME STABILITY AND GENE  
EXPRESSION

**PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.**

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

RYAN MOHAN  
DEBBIE DONG

## 1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA?  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
1. REPLICATION DEFECTIVE	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.010L 1x10 <sup>6</sup> /mL	PRODUCED IN LAB	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
MALONEY MURINE LEUKEMIA VIRUS	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
2. LABORATORY ESCHERICHIA COLI	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.5L 1x10 <sup>9</sup> cfu/mL	PROMEGA	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
3. BACULOVIRUS (INSECT VIRUS)	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.050L 3x10 <sup>7</sup> pfu/mL	PRODUCED IN LAB	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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  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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	MOUSE EMBRYO FIBROBLASTS	2006-086-08
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	HELA, HELAS3, MCF7	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	NIH3T3	ATCC
Non-human primate	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	COS7	ATCC
Other (specify)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	SF9 (INSECT)	ATCC

\*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  3

### 3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	BRAIN TUMOUR TISSUE BANK	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3
Human Organs or Tissues (preserved)	ONTARIO TUMOUR BANK	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3

### 4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5X, BL21 E. coli	PCMX, PET PLNXC, PGEX	CLONTECH NOVAGEN GE LIFE SCIENCES	THYMINE DNA GLYCOSYLASE AND	POTENTIAL CYTOTOXICITY DUE TO EXPRESSION

\* Please attach a Material Data Sheet or equivalent if available. ASSORTED TRANSCRIPTION FACTORS OF MAMMALIAN PROTEIN

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
MALONEY MURINE LEUKEMIA VIRUS	PLNCX	CLONTECH	THYMINE DNA GLYCOSYLASE	POTENTIAL CELL CYCLE AND DNA REPAIR DEFECTS

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective?  YES  NO

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

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

### 5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? \_\_\_\_\_

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

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

### 6.0 Animal Experiments

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

6.2 Name of animal species to be used \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_

6.4 Will any of the agents listed be used in live animals  YES, specify: \_\_\_\_\_  NO





**13.0 Containment Levels**

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

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, permit # if on-campus BIO-UWO-0151  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

**14.0 Procedures to be Followed**

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE  Date: May 11/09

**15.0 Approvals**

UWO Biohazard Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

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

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

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:

In vertebrate genomes the cytosine-guanine (CpG) dinucleotides mutate at a high rate due to spontaneous deamination of cytosine which results in CpGs occurring at only one quarter of the expected frequency. The CpG mutation rate is increased by methylation of cytosine, which promotes deamination to form thymine. Consequently, in humans the incidence of 5-methylcytosine to thymine mutations is 10 to 50-fold higher than other base transitions. As a result, more than one third of germline point mutations in genetic diseases and a large fraction of somatic mutations in cancer are due to CpG hypermutability. Cytosine methylation also has an integral role in transcriptional regulation and the promoter regions of genes are enriched in CpG. The integrity of CpGs in these regions is safeguarded by thymine DNA glycosylase which removes aberrant thymine and uracil from G:T and G:U mismatches produced by deamination of 5-methylcytosine and cytosine, respectively. TDG also functionally interacts with transcription factors and epigenetic regulators and serves as a transcriptional cofactor. In previous studies, we have shown that TDG associates directly with protein acetylases CBP (CREB-binding protein) and p300, and the resulting complexes participate in both DNA repair and transcriptional regulation. TDG strongly activates CBP/p300-dependent transcription and reciprocally serves as a substrate for CBP/p300 acetylation. We have shown that two Small Ubiquitin-like Modifier-1 (SUMO-1) protein-binding motifs in TDG are essential for transcriptional stimulation and regulation of subnuclear localization. Moreover, sumoylation of TDG abrogates DNA binding and CBP/p300 association. Recently, our studies have revealed that protein kinase C  $\alpha$  (PKC $\alpha$ ) interacts with the amino-terminal region of TDG and phosphorylates serine residues adjacent to lysines acetylated by CBP/p300. We found that acetylation and phosphorylation are mutually exclusive and their interplay dramatically alters the DNA mismatch processing functions. Remarkably, acetylation selectively prevents G:T processing by abrogating DNA interactions. In contrast, phosphorylation does not alter substrate interactions but may preserve G:T processing *in vivo* by preventing CBP-mediated acetylation. These findings highlight the importance of posttranslational modifications (PTMs) in regulating TDG and suggest that their interplay *in vivo* is critically important in CpG maintenance and epigenetic regulation. Consistent with the important roles of TDG, we have demonstrated that disruption of the *Tdg* gene causes embryonic lethality in mice. In view of the instability of CpG dinucleotides, and the link between aberrant genomic methylation and cancer, elucidation of the physiological roles and regulation of TDG will further our understanding of the molecular processes deregulated in cancer cells. I plan to further investigate the biological functions of TDG by pursuing the following objectives.

**Aim I. Molecular analysis of the genome maintenance and gene regulatory functions of TDG:**

To further elucidate the genome surveillance and transcriptional roles of TDG, we will map the *in vivo* binding sites in the entire genome using chromatin immunoprecipitation on chip (ChIP-chip) assays. We will also use ChIP assays to decipher the mechanisms that target TDG to promoters and determine the role of PTMs in regulating the CpG maintenance and transcriptional functions.

**Aim II. Characterization of TDG posttranslational modifications in normal and transformed cells:** We will employ PTM-specific antibodies and 2-D polyacrylamide gel electrophoresis to investigate the physiological context for these modifications and establish whether alterations in PTMs are associated with different types of human cancer.

**Aim III. Elucidation of the developmental and physiological roles of TDG:** We will continue our analysis of *Tdg* null mice and identify potential causes of embryonic lethality by characterizing tissue, cellular and gene expression defects. Additionally, to permit the analysis of cancer susceptibility resulting from TDG deficiency, we will generate mice with a conditional *Tdg* allele that can be ablated in a temporal/spatial specific manner.

These investigations will contribute to a better understanding of the molecular events that regulate genome stability and should uncover new molecular tools for the diagnostic and therapeutic management of cancer.



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## Cell Biology

**ATCC® Number:** CCL-2™ [Order this Item](#)

**Designations:** HeLa

**Biosafety Level:** 2 [CELLS CONTAIN PAPOVAVIRUS ]

**Medium & Serum:** [See Propagation](#)

**Organism:** *Homo sapiens* (human)

**Price:** \$256.00

**Depositors:** WF Scherer

**Shipped:** frozen

**Growth Properties:** adherent

**Morphology:** epithelial



**Source:** **Organ:** cervix  
**Disease:** adenocarcinoma  
**Cell Type:** epithelial

**Cellular Products:** keratin  
Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]

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

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**Applications:** transfection host ( [21491] [Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

**Virus Susceptibility:** screening for Escherichia coli strains with invasive potential [21447] [21491]  
Human adenovirus 3  
Encephalomyocarditis virus  
Human poliovirus 1  
Human poliovirus 2  
Human poliovirus 3

**Reverse Transcript:** negative

**DNA Profile (STR):** Amelogenin: X  
CSF1PO: 9,10  
D13S317: 12,13.3  
D16S539: 9,10  
D5S818: 11,12  
D7S820: 8,12  
THO1: 7  
TPOX: 8,12  
vWA: 16,18

**Cytogenetic Analysis:** Modal number = 82; range = 70 to 164.

There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature. HeLa Marker Chromosomes: One copy of M1, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.

**Isoenzymes:** G6PD, A

**Age:** 31 years adult

**Gender:** female

<b>Ethnicity:</b>	Black
<b>HeLa Markers:</b>	Y
<b>Comments:</b>	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
<b>Propagation:</b>	<b>ATCC complete growth medium:</b> 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%. <b>Atmosphere:</b> air, 95%; carbon dioxide (CO <sub>2</sub> ), 5% <b>Temperature:</b> 37.0°C
<b>Subculturing:</b>	<b>Protocol:</b> <ol style="list-style-type: none"> <li>1. Remove and discard culture medium.</li> <li>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.</li> <li>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.</li> <li>4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.</li> <li>5. Add appropriate aliquots of the cell suspension to new culture vessels.</li> <li>6. Incubate cultures at 37°C.</li> </ol> <p><b>Subcultivation Ratio:</b> A subcultivation ratio of 1:2 to 1:6 is recommended <b>Medium Renewal:</b> 2 to 3 times per week</p>
<b>Preservation:</b>	<b>Freeze medium:</b> Complete growth medium supplemented with 5% (v/v) DMSO <b>Storage temperature:</b> liquid nitrogen vapor phase
<b>Related Products:</b>	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 derivative:ATCC CCL-2.3 derivative:ATCC CCL-2.1 derivative:ATCC CCL-2.2
<b>Bioreactive Factors:</b>	<b>Growth Factors:</b> T cell growth factor (TCGF)
<b>References:</b>	21447: American Public Health Association. Compendium of methods for the microbiological examination of foods. 3rd ed. Washington, DC: American Public Health Association; 1992. 21491: AOAC International Invasiveness by Escherichia coli of mammalian cells, microbiological method. Gaithersburg, MD:AOAC International;AOAC "Official Methods of Analysis of the AOAC International" 982.36. 21626: Baldi A, et al. Genomic structure of the human retinoblastoma-related Rb2/p130 gene. Proc. Natl. Acad. Sci. USA 93: 4629-4632, 1996. PubMed: 8643454 22148: Gey GO, et al. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. 12: 264-265, 1952. 22263: Chen TR. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. Cytogenet. Cell Genet. 48: 19-24, 1988. PubMed: 3180844 22766: Boshart M, et al. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 3: 1151-1157, 1984. PubMed: 6329740 22767: Schneider-Gadicke A, Schwarz E. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J. 5: 2285-2292, 1986. PubMed: 3023067 22919: Schwarz E, et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature 314: 111-114, 1985. PubMed: 2983228 22995: Pater MM, Pater A. Human papillomavirus types 16 and 18 sequences in carcinoma cell lines of the cervix. Virology 145: 313-318, 1985. PubMed: 2992153 23180: Yee C, et al. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am. J. Pathol. 119: 361-366, 1985. PubMed: 2990217 23324: Scheffner M, et al. The state of the p53 and retinoblastoma genes in human cervical carcinoma cell lines. Proc. Natl. Acad. Sci. USA 88: 5523-5527, 1991. PubMed: 1648218 25915: Jones HW Jr., et al. George Otto Gey. (1899-1970). The HeLa cell and a reappraisal of its origin. Obstet. Gynecol. 38: 945-949, 1971. PubMed: 4942173 25919: Scherer WF, Hoogasian AF. Preservation at subzero temperatures of mouse fibroblasts (strain L) and human epithelial cells (strain HeLa). Proc. Soc. Exp. Biol. Med. 87: 480-487, 1954. PubMed: 13237281 25921: Scherer WF, et al. Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J. Exp. Med. 97: 695-710, 1953. PubMed: 13052828 26623: Fang X, et al. Lysophosphatidylcholine stimulates activator protein 1 and the c-Jun N-terminal kinase activity. J. Biol. Chem. 272: 13683-13689, 1997. PubMed: 9153219 27094: Bruder JT, Kovetski I. Adenovirus infection stimulates the Raf/MAPK signaling pathway and induces interleukin-8 expression. J. Virol. 71: 398-404, 1997. PubMed: 8985363 27345: Huber M, et al. Tyrosine phosphorylation events during coxsackievirus B3 replication. J. Virol. 71: 595-600, 1997. PubMed: 8985388 27814: Olson JK, et al. Varicella-zoster virus Fc receptor gE glycoprotein: serine/threonine and tyrosine phosphorylation of monomeric and dimeric forms. J. Virol. 71: 110-119, 1997. PubMed: 8985329 27819: Goodrum FD, Ornelles DA. The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle. J. Virol. 71: 548-561, 1997. PubMed: 8985383 28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321



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### Cell Biology

<b>ATCC® Number:</b>	<b>CCL-2.2™</b> <input type="button" value="Order this Item"/>	<b>Price:</b>	<b>\$268.00</b>
<b>Designations:</b>	HeLa S3	<b>Depositors:</b>	TT Puck
<b><u>Biosafety Level:</u></b>	2 [Cells contain human papilloma virus (HPV-18) ]	<b>Shipped:</b>	frozen
<b>Medium &amp; Serum:</b>	<a href="#">See Propagation</a>	<b>Growth Properties:</b>	adherent
<b>Organism:</b>	<i>Homo sapiens</i> (human)	<b>Morphology:</b>	epithelial
<b>Source:</b>	<b>Organ:</b> cervix <b>Disease:</b> adenocarcinoma		
<b>Cellular Products:</b>	keratin		
<b>Permits/Forms:</b>	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.		
		<b><u><a href="#">Related Cell Culture Products</a></u></b>	
<b>Isolation:</b>	<b>Isolation date:</b> 1955		
<b>Applications:</b>	transfection host ( <a href="#">Roche FuGENE® Transfection Reagents technology from amaxa</a> )		
<b>Virus Susceptibility:</b>	poliovirus 1, 2, 3; vesicular stomatitis (Indiana); encephalomyocarditis; adenovirus 5		
<b>Reverse Transcript:</b>	negative		
<b>DNA Profile (STR):</b>	Amelogenin: X CSF1PO: 9,10 D13S317: 13,3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18		
<b>Cytogenetic Analysis:</b>	A medium-sized metacentric marker is present in 100% of the cells. HeLa Markers: One copy of M1, one copy of M2, two copies of M3, and one copy of M4.		
<b>Isoenzymes:</b>	G6PD, A		
<b>Age:</b>	31 years		
<b>Gender:</b>	female		
<b>Ethnicity:</b>	Black		
<b>HeLa Markers:</b>	Y		
<b>Comments:</b>	HeLa S3 is a clonal derivative of the parent HeLa line (see ATCC <a href="#">CCL-2</a> ). S3 was cloned in 1955 by T.T. Puck, P.I. Marcus, and S.J. Cieciura. [22814] The HeLa S3 clone has been very useful in the clonal analysis of mammalian cell populations relating to chromosomal variation, cell nutrition, and plaque-forming ability. This line can be adapted to grow in suspension. [25952] The cells are positive for keratin by immunoperoxidase staining. A culture at approximately passage 400 was submitted to the American Type Culture Collection in February, 1972. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. [23180]		



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## Cell Biology

**ATCC® Number:** HTB-22™ 
**Designations:** MCF7

**Biosafety Level:** 1

**Medium & Serum:** [See Propagation](#)
**Organism:** *Homo sapiens* (human)

**Price:** \$264.00

**Depositors:** CM McGrath

**Shipped:** frozen

**Growth Properties:** adherent

**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

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[Related Cell Culture Products](#)
**Applications:** transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)

**Receptors:** estrogen receptor, expressed

**Antigen Expression:** Blood Type O; Rh+

**DNA Profile (STR):** Amelogenin: X  
 CSF1PO: 10  
 D13S317: 11  
 D16S539: 11,12  
 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

<b>Comments:</b>	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.
<b>Propagation:</b>	<b>ATCC complete growth medium:</b> 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% . <b>Atmosphere:</b> air, 95%; carbon dioxide (CO <sub>2</sub> ), 5% <b>Temperature:</b> 37.0°C
<b>Subculturing:</b>	<b>Protocol:</b> 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. <b>Note:</b> 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.  <ol style="list-style-type: none"> <li>1. Remove culture medium to a centrifuge tube.</li> <li>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.</li> <li>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.</li> <li>4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.</li> <li>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.</li> <li>6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.</li> <li>7. Incubate cultures at 37C.</li> </ol> <b>Subcultivation Ratio:</b> A subcultivation ratio of 1:3 to 1:6 is recommended <b>Medium Renewal:</b> 2 to 3 times per week
<b>Preservation:</b>	<b>Freeze medium:</b> Complete growth medium supplemented with 5% (v/v) DMSO <b>Storage temperature:</b> liquid nitrogen vapor phase
<b>Doubling Time:</b>	29 hrs
<b>Related Products:</b>	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC <a href="#">30-2003</a> recommended serum: ATCC <a href="#">30-2020</a> purified DNA: ATCC <a href="#">HTB-22D</a> purified RNA: ATCC <a href="#">HTB-22R</a> 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca <sup>++</sup> , Mg <sup>++</sup> ): ATCC <a href="#">30-2101</a> Cell culture tested DMSO: ATCC <a href="#">4-X</a>
<b>References:</b>	21405: Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. <i>Science</i> 230: 943-945, 1985. PubMed: <a href="#">3933111</a> 22871: Takahashi K, Suzuki K. Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7 cells. <i>Int. J. Cancer</i> 55: 453-458, 1993. PubMed: <a href="#">8375929</a> 23046: Brandes LJ, Hermonat MW. Receptor status and subsequent sensitivity of subclones of MCF-7 human breast cancer cells surviving exposure to diethylstilbestrol. <i>Cancer Res.</i> 43: 2831-2835, 1983. PubMed: <a href="#">6850594</a> 23079: Lan MS, et al. Polypeptide core of a human pancreatic tumor mucin antigen. <i>Cancer Res.</i> 50: 2997-3001, 1990. PubMed: <a href="#">2334903</a> 23107: Pratt SE, Pollak MN. Estrogen and antiestrogen modulation of MCF7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media. <i>Cancer Res.</i> 53: 5193-5198, 1993. PubMed: <a href="#">7693333</a> 23113: Huguet EL, et al. Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. <i>Cancer Res.</i> 54: 2615-2621, 1994. PubMed: <a href="#">8168088</a> 23217: Soule HD, et al. A human cell line from a pleural effusion derived from a breast carcinoma. <i>J. Natl. Cancer Inst.</i> 51: 1409-1416, 1973. PubMed: <a href="#">4357757</a> 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. <i>Cancer Res.</i> 57: 516-523, 1997. PubMed: <a href="#">9012484</a> 32275: Littlewood-Evans AJ, et al. The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. <i>Cancer Res.</i> 57: 5386-5390, 1997. PubMed: <a href="#">9393764</a> 32278: Komarova EA, et al. Intracellular localization of p53 tumor suppressor protein in gamma-irradiated cells is cell cycle regulated and determined by the nucleus. <i>Cancer Res.</i> 57: 5217-5220, 1997. PubMed: <a href="#">9393737</a> 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. <i>Cancer Res.</i> 57: 3478-3485, 1997. PubMed: <a href="#">9270016</a> 32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. <i>Cancer Res.</i> 57: 3562-3568, 1997. PubMed: <a href="#">9270029</a> 32344: Umekita Y, et al. Human prostate tumor growth in athymic mice: inhibition by androgens and stimulation by finasteride. <i>Proc. Natl. Acad. Sci. USA</i> 93: 11802-11807, 1996. PubMed: <a href="#">8876218</a> 32467: Zamora-Leon SP, et al. Expression of the fructose transporter GLUT5 in human breast cancer. <i>Proc. Natl. Acad. Sci. USA</i> 93: 1847-1852, 1996. PubMed: <a href="#">8700847</a> 32488: Geiger T, et al. Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice. <i>Anticancer Drug Des.</i> 13: 35-45, 1998. PubMed: <a href="#">9474241</a>



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## Cell Biology

**ATCC® Number:** CRL-1658™ [Order this Item](#)

**Price:** \$256.00

**Designations:** NIH/3T3

**Biosafety Level:** 1

**Shipped:** frozen

**Medium & Serum:** [See Propagation](#)

**Growth Properties:** adherent

**Organism:** *Mus musculus* (mouse)

**Morphology:** fibroblast



**Source:** **Organ:** embryo

**Strain:** NIH/Swiss

**Cell Type:** fibroblast fibroblast;

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

[Related Cell Culture Products](#)

**Applications:** transfection host ([Nucleofection technology from Lonza](#)  
[Roche FuGENE® Transfection Reagents](#))

**Virus Susceptibility:** Murine leukemia virus

**Age:** embryo

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

**Propagation:** **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%.

**Atmosphere:** air, 95%; carbon dioxide (CO<sub>2</sub>), 5%

**Temperature:** 37.0°C

**Growth Conditions:** The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

**Subculturing:** **Protocol:**

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

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less.

**Subcultivation Ratio:** Inoculate 3 to 5 X 10<sup>3</sup> cells/cm<sup>2</sup>

**Medium Renewal:** Twice per week

<b>Preservation:</b>	<b>Freeze medium:</b> Complete growth medium supplemented with 5% (v/v) DMSO
<b>Related Products:</b>	<b>Storage temperature:</b> liquid nitrogen vapor phase Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2002
<b>References:</b>	22370: Jainchill JL, et al. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. <i>J. Virol.</i> 4: 549-553, 1969. PubMed: <a href="#">4311790</a> 26133: Andersson P, et al. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. <i>Cell</i> 16: 63-75, 1979. PubMed: <a href="#">84715</a> 26134: Copeland NG, Cooper GM. Transfection by exogenous and endogenous murine retrovirus DNAs. <i>Cell</i> 16: 347-356, 1979. PubMed: <a href="#">222457</a> 28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. <i>J. Virol.</i> 71: 42-49, 1997. PubMed: <a href="#">8985321</a> 32372: Berson JF, et al. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. <i>J. Virol.</i> 70: 6288-6295, 1996. PubMed: <a href="#">8709256</a> 32478: Jones PL, et al. Tumor necrosis factor alpha and interleukin-1beta regulate the murine manganese superoxide dismutase gene through a complex intronic enhancer involving C/EBP-beta and NF-kappaB. <i>Mol. Cell. Biol.</i> 17: 6970-6981, 1997. PubMed: <a href="#">9372929</a> 32502: Gonzalez Armas JC, et al. DNA immunization confers protection against murine cytomegalovirus infection. <i>J. Virol.</i> 70: 7921-7928, 1996. PubMed: <a href="#">8892915</a> 32522: Siess DC, et al. Exceptional fusogenicity of chinese hamster ovary cells with murine retrovirus suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process. <i>J. Virol.</i> 70: 3432-439, 1996. PubMed: <a href="#">8648675</a> 32547: Jang SI, et al. Activator protein 1 activity is involved in the regulation of the cell type-specific expression from the proximal promoter of the human profilaggrin gene. <i>J. Biol. Chem.</i> 271: 24105-24114, 1996. PubMed: <a href="#">8798649</a> 32557: Medin JA, et al. Correction in trans for Fabry disease: expression, secretion, and uptake of alpha-galactosidase A in patient-derived cells driven by a high-titer recombinant retroviral vector. <i>Proc. Natl. Acad. Sci. USA</i> 93: 7917-7922, 1996. PubMed: <a href="#">8755577</a> 32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. <i>J. Biol. Chem.</i> 271: 4561-4568, 1996. PubMed: <a href="#">8626812</a> 32582: Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. <i>Proc. Natl. Acad. Sci. USA</i> 93: 136-140, 1996. PubMed: <a href="#">8552591</a> 32702: Cranmer LD, et al. Identification, analysis, and evolutionary relationships of the putative murine cytomegalovirus homologs of the human cytomegalovirus UL82 (pp71) and UL83 (pp65) matrix phosphoproteins. <i>J. Virol.</i> 70: 7929-7939, 1996. PubMed: <a href="#">8892916</a> 32724: Shisler J, et al. Induction of susceptibility to tumor necrosis factor by E1A is dependent on binding to either p300 or p105-Rb and induction of DNA synthesis. <i>J. Virol.</i> 70: 68-77, 1996. PubMed: <a href="#">8523594</a> 32756: Cavanaugh VJ, et al. Murine cytomegalovirus with a deletion of genes spanning HindIII-J and -I displays altered cell and tissue tropism. <i>J. Virol.</i> 70: 1365-1374, 1996. PubMed: <a href="#">8627652</a> 32905: Westerman KA, Leboulch P. Reversible immortalization of mammalian cells mediated by retroviral transfer and site-specific recombination. <i>Proc. Natl. Acad. Sci. USA</i> 93: 8971-8976, 1996. PubMed: <a href="#">8799138</a>

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## Cell Biology

**ATCC® Number:** CRL-1651™ [Order this Item](#)

**Designations:** COS-7

**Biosafety Level:** 2 [Cells Contain SV-40 viral DNA sequences ]

**Medium & Serum:** See [Propagation](#)

**Organism:** *Cercopithecus aethiops*

**Price:** \$264.00

**Depositors:** Y Gluzman

**Shipped:** frozen

**Growth Properties:** adherent

**Morphology:** fibroblast



**Source:** **Organ:** kidney  
**Cell Type:** SV40 transformed

**Cellular Products:** T antigen

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**[Related Cell Culture Products](#)**

**Applications:** transfection host ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

**Virus Susceptibility:** SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region

**Comments:** This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region. The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.

**Propagation:** **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

**Atmosphere:** air, 95%; carbon dioxide (CO<sub>2</sub>), 5%

**Temperature:** 37.0°C

**Subculturing:** **Protocol:**

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

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

**Medium Renewal:** 2 to 3 times per week

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

**Storage temperature:** liquid nitrogen vapor phase

- Related Products:** 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101  
 Cell culture tested DMSO:ATCC 4-X  
 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002  
 recommended serum:ATCC 30-2020  
 parental cell line:ATCC CCL-70
- References:** 1822: Gluzman Y. SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* 23: 175-182, 1981. PubMed: 6260373  
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## Cell Biology

<b>ATCC® Number:</b>	<b>CRL-1711™</b>	<input type="button" value="Order this Item"/>	<b>Price:</b>	<b>\$264.00</b>
<b>Designations:</b>	Sf9		<b>Depositors:</b>	G Smith, C Cherry, MD Summers
<b><u>Biosafety Level:</u></b>	1		<b>Shipped:</b>	frozen
<b>Medium &amp; Serum:</b>	<a href="#">See Propagation</a>		<b>Growth Properties:</b>	mixed: adherent/suspension
<b>Organism:</b>	Spodoptera frugiperda (fall armyworm)		<b>Morphology:</b>	epithelial



**Source:** **Organ:** ovary

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

[Related Cell Culture Products](#)

**Isolation:** **Isolation date:** 1983

**Applications:** transfection host ([Roche FuGENE® Transfection Reagents technology from amaxa](#))

**Virus Susceptibility:** Nuclear polyhedrosis virus, Autographa californica St. Louis encephalitis virus

**Age:** pupa

**Gender:** female

**Comments:** This line can be used to replicate baculovirus expression vectors. It is important to use the medium described below.

**Propagation:** **ATCC complete growth medium:** Grace's Insect Medium with L-glutamine and supplemented with:  
500 mg/L calcium chloride  
2800 mg/L potassium chloride  
3330 mg/L lactalbumin hydrolysate  
3330 mg/L yeastolate

10% Heat-inactivated fetal bovine serum (previously tested for insect cell culture)

**Atmosphere:** air, 100%

**Temperature:** 28.0°C

**Growth Conditions:** The recommended media are formulated for use without CO<sub>2</sub>. Omission of the yeastolate or lactalbumin hydrolysate will lead to poor performance by this line.

**Subculturing:** **Protocol:** Gently resuspend cells in the spent culture medium by pipetting across the monolayer or by hitting the flask against the palm of your hand (the latter is only preferable when working with larger flasks).

**Subcultivation Ratio:** A subcultivation ratio of 1:5 or greater is recommended

**Medium Renewal:** Every 2 to 3 days

**Preservation:** **Freeze medium:** culture medium, 95%; DMSO, 5%

**Storage temperature:** liquid nitrogen vapor phase

**References:** 22650: Vaughn JL, et al. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). *In Vitro* 13: 213-217, 1977. PubMed: [68913](#)

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- 33160: Yasuda H, et al. Role of the prenyl group on the G protein gamma subunit in coupling trimeric G proteins to A1 adenosine receptors. *J. Biol. Chem.* 271: 18588-18595, 1996. PubMed: [8702509](#)

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**Location of Features**

- 5' MoMuSV LTR: 1–589
- $\Psi^*$  (extended packaging signal): 659–1468  
Mutated *gag* (ATG→TAG): 1049–1051
- Neomycin resistance gene (Neo<sup>r</sup>):  
Start codon: 1512–1514; stop codon: 2304–2306
- Immediate early CMV promoter ( $P_{CMV}$ ): 2656–3393
- Multiple Cloning Site (MCS): 3465–3482
- 3' MoMuLV LTR: 3522–4115
- Col E1 origin of replication:  
Site of replication initiation: 4651
- Ampicillin resistance gene ( $\beta$ -lactamase):  
Start codon: 6271–6269; stop codon: 5413–5411

**Sequencing Primer Locations**

- pLNCX Seq/PCR Primers (#K1060-F)  
5' primer (3368–3393): 5'-AGCTCGTTTAGTGAACCGTCAGATCG-3'  
3' primer (3544–3519): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

**Propagation in *E. coli***

- Suitable host strains: DH5 $\alpha$ , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

**References**

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
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**Notes:** The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

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  - ▶ pBAC Vectors
  - ▶ pBiEx Vectors
  - ▶ pCDF & pRSF Vectors
  - ▶ pCITE Vectors
  - ▶ **pET Vectors**
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  - ▶ pETcoco Vectors
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  - ▶ pT7Blue Vectors
  - ▶ pTandem Vectors
  - ▶ pTriEx Vectors
  - ▶ SCREEN Vectors
  - ▶ T7Select Vectors
  - ▶ Discontinued Vectors

### pET *E. coli* T7 expression vectors

The pET System is the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*. Based on the T7 promoter-driven system originally developed by Studier and colleagues (1–3), Novagen's pET System has been used to express thousands of different proteins. In pET vectors, target genes are cloned under control of strong bacteriophage T7 transcription and translation signals, and expression is induced by providing a source of T7 RNA polymerase in the host cell.

Novagen's pET System has continuously expanded to offer new technologies and options for expression, and includes over 42 pET vector types, 15 different host strains and many other companion products designed for efficient detection and purification of target proteins.

1. Moffatt, B.A. and Studier, F.W. (1986) *J. Mol. Biol.* **189**, 113–130.
2. Rosenberg, A.H., Lade, B.N., Chui, D., Lin, S., Dunn, J.J., and Studier, F.W. (1987) *Gene* **56**, 125–135.
3. Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. (1990) *Meth. Enzymol.* **185**, 60–89.

pET Vectors with unique features include:

- pET-31b(+) for high yield bioproduction of peptides and small proteins
- pET-32a-c for production of soluble, active target proteins in *E. coli*
- pET-33b for production of target proteins suitable for site-specific <sup>32</sup>P-labeling
- pET-39b and 40b using Dsb tags for export and periplasmic folding of target proteins
- pET-41a-c and 42a-c with popular GST fusion tags for enhanced production and solubility
- pET-43\_1a-c designed for cloning and high-level expression of polypeptide sequences fused with the 495 aa NusA (Nus-Tag™) protein
- pET-44a-c with Nus-Tag™ sequence plus N- and C-terminal His-Tag sequences
- pET-45b(+) with amino-terminal His-Tag™ sequence and minimal extraneous sequences
- pET-46 Ek/LIC prepared vector for ligation-independent cloning, with amino-terminal His-Tag™ sequence
- pET-47b(+) through pET-50b(+) with HRV 3C Protease cleavage site for efficient fusion tag removal

Additional vectors in this table include:

- pLacI
- pLysE and pLysS

#### A Note on the Sequences

The numbering system of the pET vectors is based on that of pBR322, from which all of these vectors are derived. The sequences are given in the same orientation as the pBR322 sequence, but note that this is the **opposite** orientation of the the cloning/expression region detail maps. This should be apparent from the counter-clockwise orientation of the T7 promoter on the circle maps.

Most of the vector sequences are provided in GenBank format, with significant features indicated. To convert the sequence into other formats, first select the sequence and copy (Command-C on Mac or Ctrl-C on Windows). Next, [click here](#) to open the NIH ReadSeq web page in a new window. Paste the sequence into the input field (Command-V on Mac or Ctrl-V on PC), choose your favorite format from the dropdown menu, and hit the "submit" button.

You can access sequences, maps, ordering information, newsletter articles and technical bulletins through the following links:

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Vector	Sequence	Map	Ordering Information	Newsletter Articles	Technical Bulletins
pET-3a					
pET-3b					

## pGEX Vectors (GST Gene Fusion System)

### Technical Information

Thirteen pGEX vectors are available (see figure). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors. pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3 each encode the recognition sequence for site-specific cleavage by PreScission™ Protease (see [PreScission Protease](#)) between the GST domain and the multiple cloning site. pGEX-4T-1, pGEX-4T-2, and pGEX-4T-3 are derived from pGEX-2T and contain a thrombin protease recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX-5X-3 are derivatives of pGEX-3X and possess a factor Xa protease recognition site.

[Download the pGEX sequence map in PDF format.](#) For ASCII format please scroll down.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labeling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from heart muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labeled using protein kinase and [ $^32$ P]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T; its fusion proteins can be cleaved with thrombin.

Cleavage of pGEX-6P GST fusion proteins occurs between the Gln and Gly residues of the recognition sequence Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro<sup>(2)</sup>. Low temperature (5°C) digestion minimizes the degradation of the protein of interest. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed from the cleavage mixture simultaneously with the GST portion of the fusion protein. The pGEX-6P Expression Vectors permit convenient site-specific cleavage and simultaneous purification on Glutathione Sepharose™. The pGEX-6P series provides all three translational reading frames linked between the GST coding region and the multiple cloning site.

Collectively, the pGEX vectors provide all three translational reading frames beginning with the EcoR I restriction site. pGEX-1IT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from *lgt11* libraries.

Vector	Unformatted	Formatted	GenBank	Accession No.
pGEX-1 lambda T, 27-4805-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13849	
pGEX-2T, 27-4801-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13850	
pGEX-2TK, 27-4587-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13851	
pGEX-3X, 27-4803-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13852	
pGEX-4T-1, 27-4580-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13853	
pGEX-4T-2, 27-4581-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	<a href="#">PDF</a>	U13855
pGEX-5X-1, 27-4584-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13856	
pGEX-5X-2, 27-4585-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13857	
pGEX-5X-3, 27-4586-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U13858	
pGEX-6P-1, 27-4597-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U78872	
pGEX-6P-2, 27-4598-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U78873	
pGEX-6P-3, 27-4599-01	<a href="#">ASCII</a>	<a href="#">PDF</a>	U78874	

Click on "ASCII" to download an unformatted sequence for use by a sequence analysis program. Click on "PDF" to download a formatted sequence and restriction site table. If you prefer accessing the sequence in [GenBank](#), refer to the right-hand column for the GenBank accession number:

- **Expression:** Proteins are expressed as fusion proteins with the 26 kDa glutathione S-transferase (GST). The GST gene contains an ATG and ribosome-binding site, and is under control of the *tac* promoter. A translation terminator is provided in each reading frame. The resulting fusion protein may be purified using the GST Purification Module (27-4570-01, -02; see [GST Purification Modules](#).)
- **Enzymatic cleavage with PreScission™ Protease:** pGEX-6P-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with PreScission™ Protease. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed simultaneously with the GST portion of the fusion protein.
- **Enzymatic cleavage with thrombin:** pGEX-1 lambda T, pGEX-2T, pGEX-2TK, pGEX-4T-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with thrombin.
- **Enzymatic cleavage with factor Xa:** pGEX-3X, pGEX-5X-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with factor Xa.
- **Direct labeling *in vitro*:** pGEX-2TK allows for direct labeling of fusion proteins *in vitro* with  $^{32}$ P using the catalytic subunit of cAMP-dependent protein kinase.
- **Host(s):** *E. coli*. The plasmid provides *lac Iq* repressor.
- **Selectable marker(s):** Plasmid confers resistance to 100 µg/ml ampicillin.
- **Amplification:** Recommended.

Properties of pGEX Vectors • Induction: *tac* promoter inducible with 1-5 mM IPTG.