THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	**Gregory A. Dekaban**
SIGNATURE	
DEPARTMENT	**Molecular Brain Research Group**
ADDRESS	**Room 2214A, Robarts Research Institute**
PHONE NUMBER	**519-931-5777 ext. 24241**
EMERGENCY PHONE NUMBER(S)	**519-472-4677 (home) or 519-282-0642 (cell)**
EMAIL	**dekaban@robarts.ca**

Location of experimental work to be carried out: Building(s) **Robarts, Rms 2214, 2222 and DSB, Rm 6008**

*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: **Funding provided via Dr. Michael Rieder's grant associated with**
GRANT TITLE(S): **his GSK Chair Award. This project should be completed by January 2011.**

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

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

Kemi Adeyanju
Christy Willert

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

Summary of Project

Treatment of HIV/AIDS is characterized by medications to control the viral load as well as the plethora of diseases that are concomitant with the infection. While this has greatly improved the quality and longevity of the life of patients it comes with the added complication of adverse drug reactions (ADR) to the different classes of drugs. One of the most common is the reaction to sulphamethoxazole (SMX), which is used as prophylaxis and treatment to pneumocystis pneumonia. While SMX is the first line treatment, it has been associated with hypersensitivity ADR in up to 50% in HIV/AIDS patients compared to 3% in the general population and in HIV asymptomatic individuals. Previous studies have shown that the HIV regulatory protein Tat together with a reactive metabolite of sulphamethoxazole, SMX-HA (sulphamethoxazole-hydroxylamine), is involved in the pathogenesis of these ADRs in HIV/AIDS patients.

The object of the project is to determine what region of Tat and hence the mechanism by which HIV Tat mediates this increase in the ADR to SMX-HA. The project utilizes human cell lines expressing the HIV protein Tat or one of several C-terminal deletion mutants fused to an enhanced green fluorescent protein (EGFP) in an inducible pBIG vector. The plasmids carrying the TatGFP constructs were cloned in DH5α *E. coli* and/ or TOP10 cells. Stably transfected cell lines were established by transfecting the pBIG plasmid vectors expressing Tat or one of several C-terminal deletion mutants into the human T cell line Jurkatas well as the Cos7 and HEK lines. The cell lines were characterized by the use of real-time PCR and western blots to quantify the production of mRNA and protein respectively. We have demonstrated that differential expression of Tat is associated with differential cellular sensitivity to SMX-HA. Currently, 2-dimensional gel electrophoresis is being employed to look at the differential haptenation of the cellular proteins in the presence and absence of HIV Tat and its mutants.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓ YES O NO
(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 K12 Strains **	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1 litre	Commercial	✓ 1 O 2 O 3
HIV**	✓ Yes O No	✓ Yes O No	✓ Yes O No	20 ml	AIDS Repository	O 1 O 2 ✓ 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.
** **See Appendix I**

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	O Yes ✓ No		**Not applicable**
Rodent	O Yes ✓No		
Non-human primate	O Yes ✓No		
Other (specify)	O Yes ✓No		

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	✓Yes O No	**H9, Molt-3, CEM-SS Jurkat E6.1**	**AIDS Repository or ATCC**
Rodent	O Yes ✓No		
Non-human primate	✓Yes O No	**Cos - 7**	**ATCC**
Other (specify)	O Yes O No		

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		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? O YES, complete table below O NO

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

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO **N/A**

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

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

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

8.5 How much of the toxin is stored*? _____

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

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

9.0 Insects Requiring CFIA Permits

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

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

10.0 Plants Requiring CFIA Permits

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

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

10.3 What is the origin of the plant? _____

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

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

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

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

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

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

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

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

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

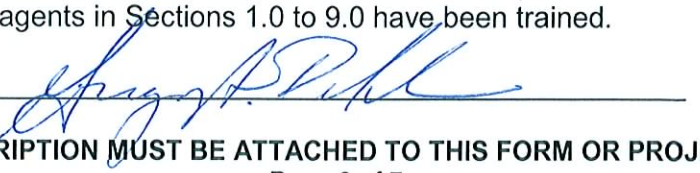
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE 

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

13.0 Containment Levels

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

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

✓YES, permit # if on-campus Awaiting certification by Health Canada

O NO, please certify

O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE: _____ Date: May 10, 2010

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

Work in level 3 to be done and when Staff Health physician on UWO Campus.

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

Fellow SOP's, wash/scrub wound with appropriate disinfectant/detergent, then go immediately to Staff/Faculty Health.

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

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

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

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

Special Conditions of Approval:

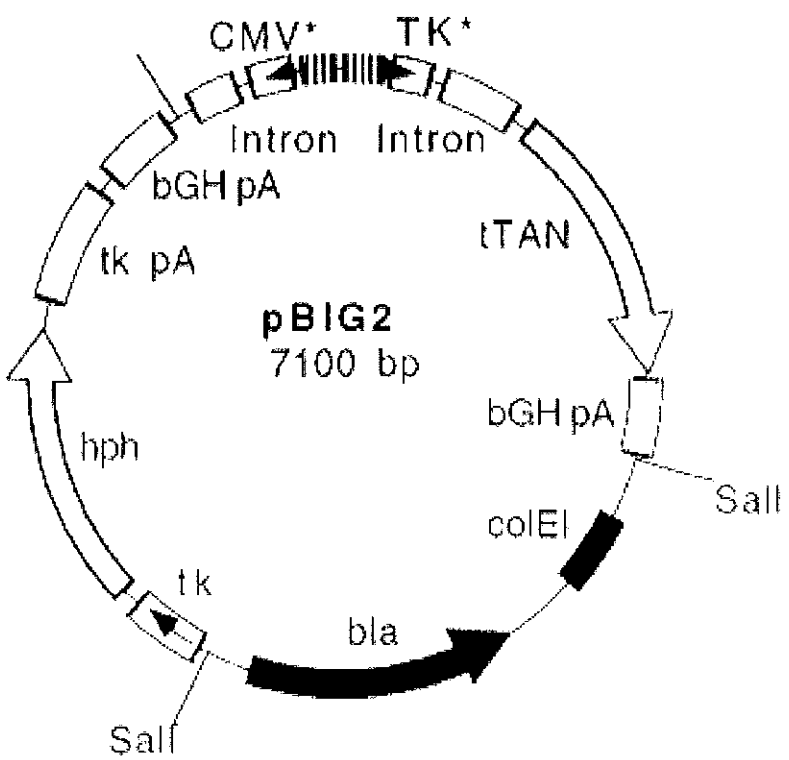
*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Summary of Project

The project utilizes human cell lines expressing the HIV protein Tat fused to a green fluorescent protein in an inducible pBIG vector. The plasmids carrying the TatGFP constructs were cloned in DH5α *E. coli* and/ or TOP10 cells and cell lines were established by transfecting the pBIG plasmid vectors expressing Tat into the human T cell line Jurkatas well as the Cos7 and HEK lines. The cell lines were characterized by the use of real-time PCR or western blots to quantify the production of mRNA and protein respectively.

In this study we use the Jurkat T cell lines expressing Tat to assess cellular toxicity in the presence of SMX-HA(sulphamethoxazole hydroxylamine) and SMX. The Cos7 and HEK cell lines were used for confocal imaging.

Below is the pBIG plasmid map:



Appendix I

Plasmids:

1.	pBIG	doxycycline inducible expression vector base for cloning (see following page)
2.	pBIG GFP	expresses EGFP
3.	pBIG Tat48GFP	expresses N-terminal half of Tat fused to EGFP
4.	pBIG Tat72GFP	expresses first 72 amino acids of Tat fused to EGFP
5.	pBIG Tat86GFP	expresses first 86 amino acids of Tat fused to EGFP
6.	pBIG Tat101GFP	expresses the full length Tat fused to EGFP
7.	pBIG Tat101	expresses the full length Tat
8.	pBIG TatΔGFP	expresses the full length Tat minus the region that contains the nuclear localisation sequence and sequence that allows Tat to cross membranes

Parent and transfected eukaryotic cell lines:

1.	Jurkat E6.1	Supplied by ATCC
2.	Jurkat pBIG GFP	Created in Dr. Dekaban's lab.
3.	Jurkat pBIG Tat48GFP	Created in Dr. Dekaban's lab.
4.	Jurkat pBIG Tat72GFP	Created in Dr. Dekaban's lab.
5.	Jurkat pBIG Tat86GFP	Created in Dr. Dekaban's lab.
6.	Jurkat pBIG Tat101GFP	Created in Dr. Dekaban's lab.
7.	Jurkat pBIG Tat101	Created in Dr. Dekaban's lab.
8.	Jurkat pBIG TatΔGFP	Created in Dr. Dekaban's lab.
9.	Cos 7	Supplied by ATCC
10.	Cos pBIG Tat48GFP	Created in Dr. Dekaban's lab.
11.	Cos pBIG Tat72GFP	Created in Dr. Dekaban's lab.
12.	Cos pBIG Tat101GFP	Created in Dr. Dekaban's lab.
13.	Cos pBIG TatΔGFP	Created in Dr. Dekaban's lab.

Bacterial Cell Lines:

1.	E. coli DH5α	Supplied by Invitrogen
2.	E. coli TOP10	Supplied by Invitrogen

HIV

1.	HIV_{111B} (grows in cultured cells)	Supplied by AIDS Repository/NIAID
2.	HIV_{MN}	Supplied by AIDS Repository/NIAID
3.	HIV_{RF}	Supplied by AIDS Repository/NIAID
4.	HIV_{IIIB} grows in primary cells only	Supplied by AIDS Repository/NIAID

Consequences of Tat expression in T cells and heterologus cells:
The vectors created for use in this project express wildtype HIV Tat or C-terminal deletion mutants of Tat. Most of these vectors express Tat fused to GFP so that cellular localization can be determined and to aid in the selection of transfected cell lines. While Tat on its own can alter the transcriptome of a cell it does not induce cell transformation. It can under different circumstances be both pro and anti-apoptotic. Tat does alter the Redox state of a cell and render the cells more sensitive to toxicity induced by certain classes of drugs such as sulphonamides.

PubMed

U.S. National Library of Medicine
National Institutes of Health

pBig2 original description.

Get it Western

Display Settings: Abstract

Gene. 1999 Mar 18;229(1-2):21-9.

Efficient control of tetracycline-responsive gene expression from an autoregulated bi-directional expression vector.

Strathdee CA, McLeod MR, Hall JR.

Gene Therapy and Molecular Virology Group, The John P. Robarts Research Institute, 100 Perth Drive, London, Ont., Canada. cas@rri.on.ca

Abstract

The tetracycline-responsive expression system is based on the ability of the chimeric tTA and rtTA transactivators to stimulate specifically transcription from a companion synthetic CMV* or TK* promoter element, and can provide tightly regulated gene expression that can be induced up to five orders of magnitude in cultured cells and transgenic mice. A major problem with the system is that high level expression of the tTA or rtTA transactivators causes cellular toxicity. Under conditions of prolonged expression this results in selective pressure against the stable incorporation of vectors expressing the tTA or rtTA transactivators, and makes the generation of stable cell lines and transgenic mice problematic. In this report we describe the development of a set of autoregulated bi-directional expression vectors in which the weaker TK* promoter is used to direct expression of the rtTA or tTA transactivator and the stronger CMV* element is used to direct cDNA expression. In this format the transactivator and response elements are encoded on the same vector, which simplifies the system and ensures that gene expression is effectively skewed in favor of the cDNA while maintaining a continuously low level of transactivator expression. We find that such an autoregulated system works equally well for both the tTA and rtTA transactivators, provided that they contain a nuclear localization signal. Similar to other versions of the tetracycline-responsive expression system, gene expression is tightly regulated and can be efficiently switched between the off and on expression states by doxycycline. In contrast with other tetracycline-responsive systems, however, expression of the rtTA and tTA transactivators from the autoregulated TK* promoter is low enough such that there is no cellular toxicity associated with either expression state. By incorporating a selectable marker into these vectors, all of the components required for using the system are now contained on a single plasmid construct, and we find that this format provides a more reliable and greatly simplified method for the generation of stable cell lines.

PMID: 10095100 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

LinkOut - more resources

SECTION III - DISSEMINATION

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Human Immunodeficiency Virus***

SYNONYM OR CROSS REFERENCE: HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV

CHARACTERISTICS: Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase

SECTION II - HEALTH HAZARD

PATHOGENICITY: Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency

EPIDEMIOLOGY: First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

INCUBATION PERIOD: Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years

COMMUNICABILITY: Period of communicability extends from asymptomatic period through appearance of opportunistic diseases

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Several reverse transscriptase and protease inhibitors now licensed

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PHYSICAL INACTIVATION: Effectiveness of 56·C - 60·C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56·C before serologic testing reduces residual infectivity to below detectable levels

SURVIVAL OUTSIDE HOST: Drying in environment causes rapid (within several hours) 90-99%

reduction in HIV concentration

SECTION V - MEDICAL

SURVEILLANCE: Serological monitoring for evidence of HIV infection

FIRST AID/TREATMENT: Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV

IMMUNIZATION: None available

PROPHYLAXIS: Experimental prophylaxis with AZT/DDI or other appropriate drug

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 5 reported laboratory acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwide

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products

PRIMARY HAZARDS: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and splashing infected materials - virus should be presumed in/on all equipment and devices coming in direct contact with infected materials

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and non-cultured procedures (primary containment devices may be indicated eg. biological safety cabinets) and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV; Biosafety level 3 practices, containment equipment and facilities for all work culturing HIV

PROTECTIVE CLOTHING: Gloves should be worn when handling potentially infectious specimens, cultures or tissues; laboratory coats, gowns or suitable protective clothing should be worn

OTHER PRECAUTIONS: Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal - steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: September 1996 **Prepared by:** Office of Biosafety

LCDC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 1997-10-11

Cell Biology

ATCC® Number:	**CCL-119™**	Order this Item	Price:	**$256.00**

Designations:	CCRF-CEM [CCRF CEM]
Depositors:	GE Foley
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Organ:** peripheral blood **Disease:** acute lymphoblastic leukemia **Cell Type:** T lymphoblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** November, 1964
Applications:	transfection host (Nucleofection technology from Lonza)
Tumorigenic:	Yes
Reverse Transcript:	negative
Antigen Expression:	CD3; Homo sapiens, expressed CD4; Homo sapiens, expressed CD5; Homo sapiens, expressed CD7; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,11 D13S317: 11,12 D16S539: 10,13 D5S818: 12,13 D7S820: 9,13 THO1: 6,7 TPOX: 8 vWA: 17,19

Note: CEM-SS are a special derivative of the CEM cell line isolated by Dr. P. Nara. See attached following this ATCC attachment.

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

Cytogenetic Analysis:	modal number = 47; range = 41 to 95. Fifty karyotypes showed no consistent loss or gain of particular chromosomes. Twenty-eight percent of the cells with 45 chromosomes were C-; 53% of all cells had an extra D and 35% had an extra F. Only N1 and N18 were not affected by gain or loss. No marker chromosomes noted.
Isoenzymes:	ADA, 1 ES-D, 1 G6PD, B GLO-I, 1 PEP-D, 1 PGD, C PGM1, 1 PGM3, 0
Age:	4 years juvenile
Gender:	female
Ethnicity:	Caucasian
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: fetal bovine serum to a final concentration of 10%. **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 to 3 X 10 exp5 viable cells/ml. Maintain cell density between 2 to 3 X 10 exp5 and 1 to 2 X 10 exp6 viable cells/ml. **Medium Renewal:** Add fresh medium (20% to 30% by volume) every 2 to 3 days
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 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-2264 derivative:ATCC CRL-2265 derivative:ATCC CRL-8993 derivative:ATCC TIB-195

References:

22788: Foley GE, et al. Continous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer 18: 522-529, 1965. PubMed: 14278051
23337: Sandstrom PA, Buttke TM. Autocrine production of extracellular catalase prevents apoptosis of the human CEM T-cell line in serum-free medium. Proc. Natl. Acad. Sci. USA 90: 4708-4712, 1993. PubMed: 8506323
25967: Adams RA. Formal discussion: the role of transplantation in the experimental investigation of human leukemia and lymphoma. Cancer Res. 27: 2479-2482, 1967. PubMed: 4170381
26284: Uzman BG, et al. Morphologic variations in human leukemic lymphoblasts (CCRF-CEM cells) after long-term culture and exposure to chemotherapeutic agents. A study with the electron microscope. Cancer 19: 1725-1742, 1966. PubMed: 5224274
26293: Adams RA, et al. Leukemia: serial transplantation of human leukemic lymphoblasts in the newborn Syrian hamster. Cancer Res. 27: 772-783, 1967. PubMed: 4295047
32913: Miranda L, et al. Isolation of the human PC6 gene encoding the putative host protease for HIV-1 gp160 processing in CD4+ T lymphocytes. Proc. Natl. Acad. Sci. USA 93: 7695-7700, 1996. PubMed: 8755538

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NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
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Germantown, MD 20874
USA

Phone: 240-686-4740
Fax: 301-515-4015
aidsreagent.org

DATA SHEET

Reagent	CEM-SS
Catalog Number	776
Lot Number	15 070569
Provided	1.3×10^7 cells/mL. Viability is 96%.
Propagation Medium	RPMI 1640, 89%; PSN antibiotics (Gibco), 1%; fetal bovine serum, 10%.
Freeze Medium	RPMI 1640, 66%; fetal bovine serum, 27%; DMSO, 7%.
Growth Characteristics	These cells double approximately every 1-2 days and grow as a suspension of single or small (3-10 cell) aggregates. The cells are optimally maintained on a rocker platform or roller bottle apparatus and can be split at 1:20 one to two times per week.
Morphology	Generally a round, individual, slightly refractile cell population that occasionally forms small aggregates as observed under normal culture conditions. Small numbers of individual highly refractile karyocytomegalic cells may also be observed.
Sterility	Negative for bacteria, mycoplasma, and fungi.
Description	Human T4-lymphoblastoid cell line initially derived by G.E Foley et al. and biologically cloned by P.L. Nara et al.
Special Characteristics	These cells have been cloned for both poly-L-lysine induced adherence to microtiter plates and viral-induced syncytial/fusigenic sensitivity following infection with either cell-free or cell-associated HIV-1 and HIV-2. Cells are negative for any virus including human retroviruses as determined by electron microscopy and reverse transcriptase analysis. They can be used for virus production, aspects of HIV-1 cell fusion and molecular biology studies and for the analysis of infectivity, antiviral agents and neutralizing antibodies in the assays referenced below.

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Recommended Storage

Liquid nitrogen.

Contributor

Dr. Peter L. Nara.

References

Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18**:522-529, 1965.

Nara PL, Hatch WC, Dunlop NM, Robey WG, Fischinger PJ. Simple, rapid quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *AIDS Res Hum Retroviruses* **3**:283-302, 1987.

Nara PL, Fischinger PJ. Quantitative infectivity assay for HIV-1 and -2. *Nature* **332**:469-470, 1988.

NOTE

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM-SS (Cat# 776) from Dr. Peter L. Nara." Please include the references cited above in any publications.

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.

Cell Biology

ATCC® Number:	**CRL-1552™**	Order this Item	Price:	**$323.00**

Designations:	MOLT-3
Depositors:	J Minowada
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Organ:** peripheral blood **Disease:** acute lymphoblastic leukemia **Cell Type:** T lymphoblast;
Cellular Products:	terminal deoxynucleotidyl transferase (TdT) activity is high [22735]
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:	CD1 (65%), CD4 (66%), CD5 (97%), CD7 (97%)
Cytogenetic Analysis:	This is a hypertetraploid human cell line. The modal chromosome number was 97, occurring in 48% of cells. The rate of cells with higher ploidies was 2%. Three markers were common to all cells. They are: ?del(6) (q21), t(7;7), and an unidentifiable M5. The der(6) generally had three copies per cell, and the i(7q), two copies. The majority of normal chromosomes had 4 copies per cell. N8, N17, N19 and N20 had more than 4 copies in many cells. The X and Y chromosomes were two copies each per cell. Neither HSR chromosomes nor DM's were detected.
Isoenzymes:	AK-1, 0 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 0 PGM1, 1 PGM3, 0
Age:	19 years
Gender:	male

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Comments:	No immunoglobulin or Epstein-Barr virus is detectable. [22524] This line was established from cells taken from a patient in relapse. [22524] The patient had received prior multidrug chemotherapy. [22524] MOLT-3 was derived from the same patient as the MOLT-4 cell line (ATCC CRL-1582). [22524]
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: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures are maintained by addition or replacement of fresh medium. Establish new cultures at 5 X 10(5) viable cells/ml and subculture at between 1 and 2 X 10(6) cells/ml. **Medium Renewal:** Every 3 to 4 days
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 30-2001 recommended serum:ATCC 30-2020 derived from same individual:ATCC CRL-1582
References:	22524: Minowada J, et al. Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J. Natl. Cancer Inst. 49: 891-895, 1972. PubMed: 4567231 22735: Mertelsmann R, et al. T-cell growth factor (interleukin 2) and terminal transferase activity in human leukemias and lymphoblastic cell lines. Blut 43: 99-103, 1981. PubMed: 6942897

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Cell Biology

ATCC® Number:	**HTB-176™** Order this Item	Price:	**$268.00**

Designations:	H9 [derivative of HuT 78]
Depositors:	RC Gallo, M Popovic
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast 
Source:	**Disease:** lymphoma **Cell Type:** cutaneous T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-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.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Receptors:	interleukin 2 (IL-2)
Virus Susceptibility:	human immunodeficiency virus 1 (HIV-1, also known as HTLV-III or LAV)
Tumorigenic:	Yes
Antigen Expression:	CD4; HLA A1, B62, C3, DR4, DQ3
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11 D13S317: 8,12 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9 TPOX: 8,9 vWA: 14,15

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Cytogenetic Analysis:	This is a near triploid cell line (modal number = 69; range = 58 to 74). The frequency of higher ploidies is 2.5%. The line has an extremely complex karyotype with nearly 60% of the chromosomes in each cell being structurally altered marker chromosomes., Among the markers are t(3p4q), t(5q6q), t(5p6p), i(18q), i(18p); t(4q7p), and del(7)(q32). The first four of these are usually paired. Normal N4, N5, N6, N7, N10, N13, N18, N19, N20 an X are absent.
Isoenzymes:	AK-1, 0 ES-D, 1 G6PD, B GLO-I, 1 Me-2, 0 PGM1, 1 PGM3, 0
Age:	53 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The H9 cell line is a clonal derivative of the Hut 78 cell line (see ATCC TIB-161). The H9 clone was selected for permissiveness for HIV-1 replication, and has been used to isolate and propagate HIV-1 from the blood of patients with acquired immunodeficiency syndrome (AIDS) and pre-AIDS conditions.
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: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Medium Renewal:** Every 2 to 4 days Cultures can be maintained by addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension in fresh medium at 5 x 10 exp5 viable cells/ml. Maintain cultures at cell concentrations between 5 X 10 exp5 and 2 X 10 exp6 viable cells/ml. Do not allow cell concentration to exceed 3 X 10 exp6 cells/ml.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 parental cell line:ATCC TIB-161

References:

1140: Gootenberg JE, et al. Human cutaneous T cell lymphoma and leukemia cell lines produce and respond to T cell growth factor. J. Exp. Med. 154: 1403-1418, 1981. PubMed: 6975346
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Cell Biology

ATCC® Number:	**TIB-152™**	Order this Item	Price:	**$272.00**
Designations:	Jurkat, Clone E6-1			
Depositors:	A Weiss			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	suspension			
Organism:	*Homo sapiens* (human)			
Morphology:	lymphoblast 			
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;			
Cellular Products:	interleukin-2 (interleukin 2, IL-2) [1609]			
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:	T cell antigen receptor, expressed			
Antigen Expression:	CD3; Homo sapiens, expressed			
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 8,12 D16S539: 11 D5S818: 9 D7S820: 8,12 THO1: 6,9.3 TPOX: 8,10 vWA: 18			
Cytogenetic Analysis:	This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.			
Gender:	male			

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Comments:	This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609] The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530] Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609] The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]
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: 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 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml. **Interval:** Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 derivative:ATCC CRL-1990 derivative:ATCC CRL-2063 derivative:ATCC TIB-153

References:

1609: Weiss A, et al. The role of T3 surface molecules in the activation of human T cells: a two-stimulus requirement for IL-2 production reflects events occurring at a pre-translational level. J. Immunol. 133: 123-128, 1984. PubMed: 6327821
23430: Gillis S, Watson J. Biochemical and biological characterization of lymphocyte regulatory molecules. V. Identification of an interleukin 2-producing human leukemia T cell line. J. Exp. Med. 152: 1709-1719, 1980. PubMed: 6778951
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Cell Biology

ATCC® Number: CRL-1651™ Order this Item
Price: $269.00

Designations: COS-7

Depositors: Y Gluzman

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

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Cercopithecus aethiops*

Morphology: fibroblast

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

Cellular Products: T antigen

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

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

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 (CO2), 5%
Temperature: 37.0°C

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Subculturing:

Protocol:

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

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

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

Related Products:

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
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:

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32447: Fernandez LM, Puett D. Lys583 in the third extracellular loop of the lutropin/choriogonadotropin receptor is critical for signaling. J. Biol. Chem. 271: 925-930, 1996. PubMed: 8557706
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32568: Lee JH, et al. The proximal promoter of the human transglutaminase 3 gene. J. Biol. Chem. 271: 4561-4568, 1996. PubMed: 8626812
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