**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 | Dr. M.J. Rieder |
| SIGNATURE | |
| DEPARTMENT | Paediatrics |
| ADDRESS | Univ. of Western Ontario, RRI, Rm. # 2226 |
| PHONE NUMBER | 519 931 5777 x 24209 |
| EMERGENCY PHONE NUMBER(S) | 519 851 6027 |
| EMAIL | mrieder@uwo.ca |

Location of experimental work to be carried out: Building(s) DSB Room(s) 6002-6012

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

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): Mechanisms of ADRs in HIV infection and AIDS

**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 Adeyonju

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

**1.0 Microorganisms**

1.1 Does your work involve the use of biological agents? ✓ YES O NO
(including but not limited to 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 |
|---|---|---|---|---|---|---|
| HIV HIB, rf, mn | ✓ Yes O No | O Yes ✓ No | O Yes ✓ No | 50 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 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 3 |

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

**2.0 Cell Culture**

2.1 Does your work involve the use of cell cultures? ✓ YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

| Cell Type | Is this cell type used in your work? | Source of Primary Cell Culture Tissue | AUS Protocol Number |
|---|---|---|---|
| Human | ✓ Yes O No | (PBL) Peripheral Blood Lymphocytes | 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 | HA Molt-3, CEMss Jurkat | AIDS repository |
| Rodent | O Yes O No | | |
| Non-human primate | O Yes O No | | |
| 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 O 1 O 2 ~~O 3~~

**3.0 Use of Human Source Materials**

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

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

| **Human Source Material** | **Source/Supplier /Company Name** | **Is Human Source Material Infected With An Infectious Agent? YES/NO** | **Name of Infectious Agent (If applicable)** | **PHAC or CFIA Containment Level (Select one)** |
|---|---|---|---|---|
| Human Blood (whole) or other Body Fluid | VOLUNTEER DONORS | 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 |
|---|---|---|---|---|
| DH5α | pBig plasmids containing various HIV Tat genes either intact or with deletions | Craig Strathdee + AIDS Repository | HIV Tat & mutants | Loss of Tat functions depending on deletion |

* 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 ✓O NO

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted involving a biological agent? O YES ✓O NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 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 ✓O 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 O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES O NO
- Non-human primates O YES, please specify species _____ O NO N/A
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

## 8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ✓O 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 detais:_____ 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 ✓O 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 _____ X

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**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 BIO-UWO-0048

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: 20/10/09

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.

_____

_____

_____

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

_____

_____

**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 Biohazardous Agents- Dr MJ Rieder

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* cells and cell lines were established by incorporating the pBIG plasmid vectors expressing Tat into the human Jurkat T cell line. The cell lines were characterized by the use of real-time PCR or western blots to quantify the production of mRNA and protein respectively.

The project also involves the use of the pBIG cell lines as well as HIV-1 infected T cell lines including Molt-3, Jurkat and H9 cells to determine the viability of the cells in the presence of the molecules SMX-HA (sulphamethoxazole hydroxylamine) and SMX. This viability assay is caried out in a 96-well plate format in a biosafety cabinet.

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20301 Century Boulevard
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# DATA SHEET

| | |
|---|---|
| **Reagent** | ☣ H9/HTLV-III$_{RF}$ NIH 1983 |
| **Catalog Number** | 401 |
| **Lot Number** | 4 011617 |
| **Provided** | 1 x $10^7$ cells/vial. Viability is 92%. |
| **Cell Type** | Single cell clone derived from HUT 78. |
| **Propagation Medium** | RPMI 1640 with glutamine, 90%; fetal bovine serum, 10%. |
| **Freeze Medium** | RPMI 1640 with glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%. |
| **Growth Characteristics** | Cells grow as a single cell suspension. Maintain cells between 5 x $10^5$ and 1 x $10^6$ for optimum growth and split 1:2-1:4 every 2-3 days. When cells are first started in culture, split at 1:2 only for the first week. Please see attached instructions for infecting cells with HTLV-III$_{RF}$ and maintaining chronically infected cultures. HIV-1 p24 antigen capture assay showed a p24 level of 5 ng/ml. Since the amount of p24 is not very high, adding uninfected H9 cells (Cat. #87) to the culture can increase the virus output. |
| **Sterility** | The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative for adventitious agents in culture since 1984. Also negative for bacteria, fungi, and yeast. |
| **Special Characteristics** | This virus strain was obtained from peripheral blood lymphocytes in November 1983. It has been in continuous production in H9 cells since 1984. This strain is more cytopathic than HTLV-IIIB; a pronounced cytopathic effect can be seen in H9/HTLV-III $_{RF}$. There is approximately 10% difference in the nucleic acid sequence from HTLV-III $_{B}$. |

**HTLV-III$_{RF}$**

| | |
|---|---|
| **Recommended Storage** | Liquid nitrogen (vapor phase). |
| **Contributor** | Dr. Robert Gallo. |
| **References** | Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497-500, 1984.<br><br>Starcich BR, Hahn BH, Shaw GS, McNeely PD, Modrow S, Wolf H, Parks ES, Parks WP, Josephs SF, Gallo RC, and Wong-Staal F. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. *Cell* **45**:637-648, 1986. |
| **NOTE** | Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: H9/HTLV-III$_{RF}$ NIH 1983 from Dr. Robert Gallo." Also include the references cited above in any publications. |

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# DATA SHEET

| | |
|---|---|
| **Reagent** | H9 |
| **Catalog Number** | 87 |
| **Lot Number** | 21 098165 |
| **Provided** | 1.72 x $10^6$ cells/mL. Viability is 97%. |
| **Cell Type** | Single cell clone derived from a specific HUT 78 cell line, HT. HUT 78 is a human cutaneous T cell lymphoma derived from the peripheral blood of a patient with Sezary syndrome. |
| **Propagation Medium** | RPMI 1640, supplemented with 2 mM L-glutamine and 50 µg/ml gentamicin, 90%; fetal bovine serum, 10%. |
| **Freeze Medium** | RPMI 1640, 80%; fetal bovine serum, 10%; DMSO, 10%. |
| **Growth Characteristics** | Maintain H9 cells at 1 x $10^5$ - 1 x $10^6$ cells/ml. Split 1:2-1:4 twice weekly. H9 grows as a single cell suspension with some clumping. Morphology is mature lymphocytic. |
| **Sterility** | Negative for bacteria, fungi, and mycoplasma. |
| **Special Characteristics** | This cell line was selected for high yield permissive growth with HIV-1. |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | Dr. Robert Gallo |

**References**

Mann DL, O'Brien SJ, Gilbert DA, Reid Y, Popovic M, Read-Connole E, Gallo R, Gazdar A. Origin of the HIV-susceptible human CD4 $^{+}$ cell line H9. *AIDS Res Hum Retroviruses* **5**:253-255, 1989.

Popovic M, Read-Connole E, Gallo RC. T4 positive human neoplastic cell lines susceptible to and permissive for HTLV-III. *Lancet* **ii**:1472-1473, 1984.

Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497-500, 1984.

**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: H9 from Dr. Robert Gallo." Also include the references cited above in any publications.

**The use of the H9 cell line and other neoplastic T cell lines to produce HIV-1 is described in U.S. Patent 4,520,113.**

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# DATA SHEET

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|---|---|
| **Reagent** | ☥ H9/HTLV-IIIB NIH 1983 |
| **Catalog Number** | 400 |
| **Lot Number** | 006 060492 |
| **Provided** | 1.8 x $10^6$ cells/vial. Viability is 90%. |
| **Cell Type** | Single cell clone derived from HUT 78. |
| **Propagation Medium** | RPMI 1640 with L-glutamine, 90%; fetal bovine serum, 10%. |
| **Freeze Medium** | RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%. |
| **Growth Characteristics** | Cells grow as a single cell suspension. Maintain cells between 5 x $10^5$ and 1 x $10^6$/ml for optimum growth and split 1:2-1:4 every 2-3 days. When cells are first started in culture, split at 1:2 only for the first week. |
| **Sterility** | Negative for mycoplasma, bacteria and fungi. |
| **Special Characteristics** | Virus has high capacity for replication in T cell lines. This virus appears to be well adapted for *in vitro* *in vitro* culture in T cell lines and replicates less in fresh human macrophages. |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | Dr. Robert Gallo. |

**References**

Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224**:497-500, 1984. Popovic M, Read-Connole E, Gallo RC. T4 positive human neoplastic cell lines susceptible to and permissive for HTLV-III. *Lancetii:1472-1473, 1984. Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, Doran ER, Rafalski JA, Whitehorn EA, Baumeister K, Ivanoff L, Petteway SR Jr, Pearson ML, Lautenberger JA, Papas TS, Ghrayeb J, Chang NT, Gallo RC, Wong-Staal F. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature **313**:277-284, 1985.*

**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: H9/HTLV-IIIB NIH 1983 from Dr. Robert Gallo." Also include the references cited above in any publications. .

**Scientist at for-profit institutions or who intend commercial use of this reagent must contact Dr. Susan Ano, Office of Technology Transfer, National Institute of Health, 6011 Executive Blvd, Suite 325, Rockville, MD 20852, Tel:(301) 435-5515, Fax:(301) 402-0220, Email: anos@mail.nih.gov,Website: http://ott.od.nih.gov, before the reagent can be released.**

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# DATA SHEET

| | |
|---|---|
| **Reagent** | ☣ H9/HTLV-III$_{MN}$ NIH 1984 |
| **Catalog Number** | 402 |
| **Lot Number** | 2 95080 |
| **Provided** | 6 x $10^6$ cells/vial. |
| **Cell Type** | Single cell clone derived from HUT 78. Resembles H9/HTLV-III$_B$, with some giant cell formation. |
| **Propagation Medium** | RPMI 1640 with L-glutamine, 90%; fetal bovine serum, 10%. |
| **Freeze Medium** | RPMI 1640 with L-glutamine, 50%; fetal bovine serum, 40%; DMSO, 10%. |
| **Growth Characteristics** | Cells grow as a single cell suspension. Maintain cells between 5 x $10^5$ and 1 x $10^6$ for optimum growth and split 1:2-1:4 every 2-3 days. When cells are first started in culture, split 1:2 only for the first week. |
| **Sterility** | The cloned cell population was extensively characterized to exclude the presence of adventitious virus and mycoplasma and has been consistently negative for adventitious agents in culture since 1984. Also negative for bacteria, fungi, and yeast. |
| **Special Characteristics** | Virus transmitted into H9 cells in April 1984. |
| **Recommended Storage** | Liquid nitrogen (vapor phase). |
| **Contributor** | Dr. Robert Gallo. |

**References**

Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, Haynes BF, Palker TJ, Redfield R, Oleske J, Safai B, White G, Foster P, Markham PD. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **224**:500-503, 1984.

Shaw GM, Hahn BH, Arya SK, Groopman JE, Gallo RC, Wong-Staal F. Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. *Science* **226**:1165-1170, 1984.

**NOTE**

Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: H9/HTLV-III$_{MN}$ NIH 1984 from Dr. Robert Gallo." Also include the references cited above in any publications..

**Scientist at for-profit institutions or who intend commercial use of this reagent must contact Dr. Susan Ano, Office of Technology Transfer, National Institute of Health, 6011 Executive Blvd, Suite 325, Rockville, MD 20852, Tel:(301) 435-5515, Fax:(301) 402-0220, Email: anos@mail.nih.gov,Website: http://ott.od.nih.gov, before the reagent can be released.**

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|---|---|
| **Reagent** | CEM-TART |
| **Catalog Number** | 1944 |
| **Lot Number** | 3 |
| **Provided** | 1 vial frozen cells. |
| **Cell Type** | Transformed CEM cells expressing HIV-1 *tat* and *rev* genes. |
| **Propagation Medium** | RPMI 1640, 80%; heat-inactivated fetal bovine serum, 20%. Supplement with gentamicin at 1 µg/ml. |
| **Freeze Medium** | Fetal bovine serum, 90%; DMSO, 10%. |
| **Growth Characteristics** | Cells grow in suspension. Maintain at $0.5x10^6$ viable cells/ml. Doubling time is approximately 48 hours. Passage every 2-3 days. Viability is 85-95%, but decreases after infection with MC99IIIBΔTat-Rev. |
| **Sterility** | Negative for bacteria, fungi, and mycoplasma. |
| **Special Characteristics** | HIV-1 *tat* and *rev* genes are constitutively expressed in CEM-TART cells. This cell line can be infected by *tat* and *rev*-deficient proviral HIV-1 mutants such as HIV-1 MC99IIIBΔTat-Rev (Catalog #1943) and used to produce mutant virus that replicates only in CEM-TART cells. Infection is characterized by cytopathology, decreased CD4 expression, and production of viral structural proteins. |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | Drs. Herbert Chen, Terence Boyle, Michael Malim, Bryan Cullen, and H. Kim Lyerly. |

**References**

Chen H, Boyle JT, Malim MH, Cullen BR, Lyerly HK. Derivation of a biologically contained replication system for human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* **89**:7678-7682, 1992.

**NOTE**

Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM-TART from Drs. Herbert Chen, Terence Boyle, Michael Malim, Bryan Cullen, and H. Kim Lyerly." Also include the reference cited above in any publications.

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| | |
|---|---|
| **Reagent** | CEM-SS |
| **Catalog Number** | 776 |
| **Lot Number** | 15 070569 |
| **Provided** | $1.3 \times 10^7$ 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. |

**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.

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

# DATA SHEET

| | |
|---|---|
| **Reagent** | ☣ J1.1 |
| **Catalog Number** | 1340 |
| **Lot Number** | 5 080012 |
| **Provided** | 1.2 x $10^7$ cells/mL. Viability is 92%. |
| **Cell Type** | Derived from Jurkat. Morphology is mature lymphocytic. |
| **Propagation Medium** | RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 90%; fetal bovine serum, 10%. |
| **Freeze Medium** | Propagation medium, 92.5%; DMSO, 7.5%. |
| **Growth Characteristics** | When thawing, dilute the cells with 37°C medium dropwise, wash out the DMSO, and seed the initial culture at 1 x $10^6$ cells/ml. Passage the cells every three days to give a concentration of 1 x $10^6$ cells/ml. Cells grow in single cell suspension. Doubling time is 24 hours. |
| **Sterility** | Negative for mycoplasma, bacteria and fungi. |
| **Special Characteristics** | Latently HIV infected cell line cloned by limiting dilution from HIV-infected Jurkat cells. HIV capable of being induced with PMA or TNF.<br><br>Time in culture after cryorecovery of J1 Cells |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | Dr. Thomas M. Folks. |

**References**

Perez VL, Rowe T, Justement JS, Butera ST, June CH, Folks TM. An HIV-1-infected T cell clone defective in IL-2 production and $Ca^{2+}$ mobilization after CD3 stimulation. *J Immunol* **147**:3145-3148, 1991.

**NOTE**

Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: J1 from Dr. Thomas Folks." Also include the reference cited above in any publications.

**Scientists at for-profit institutions or who intend commercial use of this reagent must contact Tom O'Toole at email address teo1@cdc.gov and specify in the email the name of the reagent, and a description of the intended use of the reagent.**

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# DATA SHEET

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|---|---|
| **Reagent** | Jurkat-*tat* |
| **Catalog Number** | 1399 |
| **Lot Number** | 6 031746 |
| **Provided** | $1.5 \times 10^7$ cells/vial. Viability is 90%. |
| **Cell Type** | Human T-lymphocytic cell line. |
| **Propagation Medium** | RPMI 1640, 90%; fetal bovine serum, 10%; G418, 800 µg/ml. |
| **Freeze Medium** | Fetal bovine serum, 90%; DMSO, 10%. |
| **Growth Characteristics** | Maintain cells at approximately $5 \times 10^5$ cells/ml. Doubling time is 25 hours. Passage twice weekly. Jurkat-*tat* cells grow in suspension both singly and as clumps. |
| **Morphology** | Lymphocytic; very similar to parent cell line. |
| **Sterility** | Negative for mycoplasma, bacteria and fungi. |
| **Special Characteristics** | A BK virus expression vector containing HIV-1 *tat* cDNA and a neomycin resistance selection marker was used to transfect Jurkat cells. The resultant cell line stably expresses HIV-1 Tat. It is unclear whether Tat is secreted into the culture medium. These cells are $CD4^+$ and are easily infected with HIV-1. |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | Drs. Antonella Caputo, William Haseltine, and Joseph Sodroski. |

**References**

Caputo A, Sodroski JG, Haseltine WA. Constitutive expression of HIV-1 *tat* protein in human Jurkat T cells using a BK virus vector. *J Acquired Immune Defic Syndr* **3**:372-379, 1990.

**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: Jurkat-*tat* from Drs. Antonella Caputo, William Haseltine, and Joseph Sodroski." Also include the reference cited above in any publications.

**This and other stable cell lines expressing tat III is described in US Patent #4,981,790. Requests from commercial organizations must be directed to both Dr. Joseph Sodroski, Division of Human Retrovirology, JFB824, Dana Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA, 02115, and the Director, Office of Technology Transfer, Dana-Farber Cancer Institute, 44 Binney Street, Suite L660, Boston, MA 02115.**

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# DATA SHEET

| | |
|---|---|
| **Reagent** | Jurkat Clone E6-1 |
| **Catalog Number** | 177 |
| **Lot Number** | 11 070572 |
| **Provided** | $1.2 \times 10^7$ cells/vial, viability is 96%. |
| **Propagation Medium** | RPMI 1640 supplemented with 2 mM L-glutamine, 90%; fetal bovine serum, 10%; pen-strep. |
| **Freeze Medium** | Propagation medium, 90%; DMSO, 10%. |
| **Growth Characteristics** | The cells grow as a single cell suspension with occasional clumping. Passage the cells every 2-3 days to maintain a concentration of $1 \times 10^5$ - $1 \times 10^6$ cells/ml. Doubling time is less than 24 hours. |
| **Sterility** | Negative for bacteria, mycoplasma, and fungi. |
| **Description** | Human T cell leukemia. Jurkat Clone E6-1 was obtained by cloning Jurkat FHCRC at limiting dilution over macrophages. Morphology is lymphocytic. |
| **Special Characteristics** | This clone of Jurkat-FHCRC (Dr. Kendall Smith, Dartmouth) produces large amounts of IL-2 after stimulation with both PMA and antibody to T3. The cells are CD4+ and may be induced to secrete γ-interferon. |
| **Recommended Storage** | Liquid nitrogen. |
| **Contributor** | ATCC (Dr. Arthur Weiss). |

**References**

Weiss AL, Wiskocil RL, Stobo JD. 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.

**NOTE**

Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: Jurkat Clone E6-1 from Dr. Arthur Weiss." Also include the reference cited above in any publications.

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# DATA SHEET

| | |
|---|---|
| **Reagent:** | pSV2tat72 |
| **Catalog Number:** | 294 |
| **Lot Number:** | 3 060974 |
| **Provided:** | 1 ml ampicillin-resistant transformed HB101 bacteria. |
| **Cloning Site:** | HindIII–BglII |
| **Cloning Vector:** | pSV2-dhfr (Subramani, S., et al. *Mol. Cell. Biol.* **1**:854, 1981). |
| **Description:** | Produces Tat (residues 1-72) using the SV40 early promoter. Constructed by replacing the dhfr gene in pSV2-dhfr with a synthetic gene encoding Tat.<br>Plasmid Map<br>Sequence |
| **Special Characteristics:** | Contact contributor for additional information. |
| **Recommended Storage:** | -70°C. |
| **Contributor:** | Dr. Alan Frankel. |
| **References:** | Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* **55**:1189–1193, 1988. |

**NOTE:**

Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: pSV2tat72 from Dr. Alan Frankel." Also include the reference cited above in any publications.

pSV2tat72
4480 bp
750 BamHI
polyA
SV40
late
210 PstI
EcoRI 0
amp
PstI 3714
pBR322
PvuII 2164
SV40 ori
1824 HindIII
tat
224 bp
1637 BamHI
1600 BglII/BclI
(nonfunctional)
SV40 small T intron