Modification Form for Permit BIO-UWO-0230

Permit Holder: Stephen Barr

Approved Personnel
(Please stroke out any personnel to be removed)

Additional Personnel
(Please list additional personnel here)

Jenna Kelly
Sherry Xu

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	~~HIV-1~~, E. Coli (HB101), ~~SIV~~, Adeno-associated virus (replication incompetent), ~~lentivirus (HIV based)~~	
Approved Cells	Human (primary): blood. Human (established): HOS, U2OS, 143B, HeLa, Jurkat, U937, 293T, SupT1. Non-human primate (established): cos-1, Vero, cos-7, Phoenix cells, (Lentiviral) HUT78 (T cells),	CEM-GFP HSC-F
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid: Healthy volunteers	
Approved GMO	Plasmids: pcDNA, pFLAG, pCS2, pLKO.1. Vectors: NEO-loxP-3xFlag, deltaR9, VSVg, ppt, LTR, Gag, Env, accessory genes, pTetOn, pTRE2hyg-HIV protease, (tet-responsive) pLPCX (Retroviral), pLRCX	
Approved use of Animals		
Approved Toxin(s)		

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 11, 2008

Signature of Permit Holder

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

HSC-F:

This cell line is a cynomolgous T cell line from Macaca fascicularis. It was transformed using Herpesvirus saimiri. It is unknown whether the cell line is productively infected with Herpesvirus. Herpesvirus saimiri has been classified as NIH Risk Group 1. I wish to culture these cells at level 2. They will be transfected with human and non-human primate TRIM22 genes. TRIM22 is a proposed antiviral gene that we have shown blocks HIV infection. Following transfection, the cells will be fixed and imaged using confocal microscopy. I also wish to co-transfect these TRIM22 genes with and without Gag-only plasmids to measure the effect on Gag-only particle release. Gag-only particles are non-infectious (level 2).

I plan to perform SIV and/or HIV infections of these HSC-F cells (or the TRIM22-transfected HSC-F) to measure the antiviral activity of TRIM22 on SIV and HIV replication. This experiment will be performed in the level 3.

CEM-GFP

This is an indicator T cell line for quantifying HIV infection. Neomycin-resistant indicator cells. CEM-GFP can be used to monitor infection with HIV-1 (CXCR4, SI strains). Productive infection will generate green fluorescent protein (GFP) in the CEM-GFP cells.

I will use it to titre my HIV virus stocks after they are produced from transfection of 293T cells by calcium phosphate transfection with a plasmid encoding replication-competent HIV (see protocol on following page). HIV-infected CEM-GFP cells will be fixed with 2% paraformaldehyde for 1 hour and subjected to FACS analysis (using the London Regional Flow Cytometry Facility) to determine the percentage of cells that are positive for GFP.

Proc Natl Acad Sci U S A. 1997 Apr 29;94(9):4653-8.

A new reporter cell line to monitor HIV infection and drug susceptibility in vitro.

Gervaix A, West D, Leoni LM, Richman DD, Wong-Staal F, Corbeil J.

Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0679, USA.

Determination of HIV infectivity in vitro and its inhibition by antiretroviral drugs by monitoring reduction of production of p24 antigen is expensive and time consuming. Such assays also do not allow accurate quantitation of the number of infected cells over time. To develop a simple, rapid, and direct method for monitoring HIV infection, we generated a stable T-cell line (CEM) containing a plasmid encoding the green fluorescent protein (humanized S65T GFP) driven by the HIV-1 long terminal repeat. Clones were selected that displayed low constitutive background fluorescence, but a high level of GFP expression upon infection with HIV. HIV-1 infection induced a 100- to 1,000-fold increase in relative fluorescence of cells over 2 to 4 days as monitored by fluorescence microscopy, cytofluorimetry, and flow cytometry. Addition of inhibitors of reverse transcriptase, protease, and other targets at different multiplicities of infection permitted the accurate determination of drug susceptibility. This technique also permitted quantitation of infectivity of viral preparations by assessment of number of cells infected in the first round of infection. In conclusion, the CEM-GFP reporter cell line provides a simple, rapid, and direct method for monitoring HIV infectivity titers and antiretroviral drug susceptibility of syncytium-inducing strains.

Protocol for use of the CEM-GFP reporter cell line

1. Split the cell culture 1/3, 24h prior to use with viral isolates.
2. Use standard infection protocol, polybrene 2 µg/ml to enhance infection is recommended. Incubate cells at a concentration of 10^6/mL for 2h/37°C with viral isolates. Infection of 50.000 cells per well in a 96 well-plate is considered a minimun. Virus can remain in the culture and cells need not be washed. MOI as low as 0.00001 using HIV-1_{LAI} have been shown to generate a signal (see figure 1).
3. Feed cells every three days. The cells are G418 resistant (500µg/mL) but G418 should not be used during the infection assay.
4. Aliquots for GFP evaluation are taken as desired for analysis by cytofluorimetry or FACS.

Note: The reporter cell line works with HIV strains that use CXCR-4 as a receptor (Syncytium-inducing, lymphotropic isolates of HIV-1 as well as HIV-2). Both primary isolates and laboratory adapted strains have been shown to work with the reporter cell line. Passaging the cell line continuously may reduce sensitivity.

Figure 1

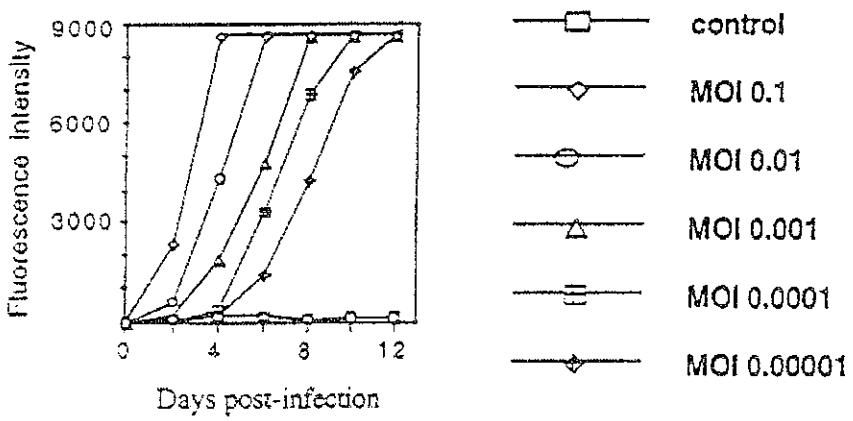


Figure 1: CEM-GFP were infected with HIV-1_{LAI} at different multiplicity of infection (MOI 0.000001 to 0.1) and intensity of fluorescence was measured by fluorimetry using a cytofluorimeter. Note that for each log reduction in inoculum an additional two days was necessary for half maximal detection (3000 arbitrary units).

Technical Data Sheet

Reagent: Cynomolgus T cell line

Clone: HSC-F

Lot: Dec 2007

Species: *Macaca fascicularis*

Description: Immortalized cell line derived by transformation of cynomolgus monkey fetal splenocytes with *Herpesvirus saimiri (1)*. IL-2-independent. Cell line kindly provided by Dr. Hirofumi Akari, Tsukuba Primate Research Center and the Health Science Research Resources Bank, Osaka, Japan

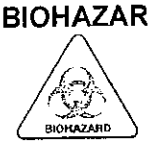
Supplied as: 10^7 cells in 90% FBS, 10% DMSO, shipped on dry ice

Medium: 90% RPMI 1640 with L-glutamine,10% FBS and penn/strep

Doubling time: 30 hours

Instructions for propagation: Cells grow as small clumps largely in suspension culture. Split cultures 1-2 times weekly as needed.

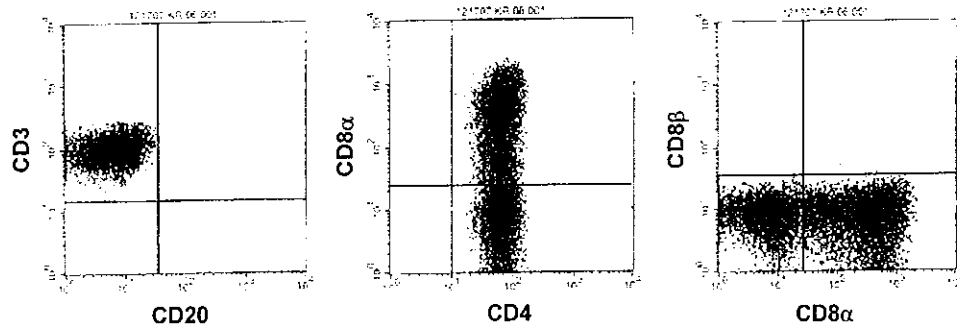
BIOHAZARD:



It is unknown whether this cell line is productively infected with *Herpesvirus saimiri. H. saimiri* has been classified as NIH Risk Group I*.

*Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. U.S Government Printing Office, Washington: 2007.

Immunophenotype: CD3+ (100%), CD4+ (100%), CD8α+ (~50%), CD8β– (0%), CD20– (0%)



References: (1) Akari H, Nam KH, Mori K, Otani I, Shibata H, Adachi A, Terao K, Yoshikawa Y. Effects of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. *Clin Immunol. 1999; 91(3):321-9.*

For Research Use Only- All reagents are to be used in accordance with the terms of the Resource registration agreement.

NIH NONHUMAN PRIMATE REAGENT RESOURCE
http://nhpreagents.bidmc.harvard.edu
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The NIH Nonhuman Primate Reagent Resource is sponsored by the National Center for Research Resources and the National Institute of Allergy and Infectious Diseases, NIH