

GHOST (3) Cell Assay

1 PRINCIPLE AND BACKGROUND

- 1.1 HIV is the etiologic agent that causes AIDS. HIV enters target cells using CD4 and a secondary chemokine receptor, these receptors are referred to as cofactors or co-receptors. These co-receptors are from the G seven-transmembrane chemokine receptor family. Some biological properties of the virus (cell tropism, cytopathicity) are determined by the co-receptor used, for example CCR5 using viruses are found predominately in the early stages of infection, are referred to as slow/low viruses and usually do not exhibit syncytia-inducing capacity. Viruses that utilize CXCR4 or that use both CCR5/CXCR4 are found in later stages of infection are referred to as rapid/high viruses and produce syncytia.
- 1.2 Ghost cells are derived from human osteo sarcoma (HOS) cells that are stably transduced with MV7neo-T4 (CD4) retroviral vector. These cells also contain the gene of the green fluorescent protein (GFP) which is driven by the HIV-2 Rod LTR. Ghost cells are engineered with antibiotic resistant selection to stably express CD4, the primary receptor used for HIV and SIV infection and one or more of the co-receptors that are required for infection. Use of high levels of antibiotics ensures the stability and expression of the CD4 and the co-receptor.
- 1.3 Viral entry activates Tat and the subsequent transcription drives the GFP expression. This expression of GFP allows for the detection of viral infection with a fluorescent microscope. Cells that are infected will glow green when viewed with a fluorescent microscope. Expression of GFP occurs soon after infection which allows evaluation within two to three days post infection. This ease of use and short duration to evaluation are the main advantages of the GHOST (3) cell assay.
- 1.4 These cells can be used to titer virus, determine the phenotypic properties and in drug/neutralization studies.

2. SPECIMEN REQUIREMENTS

- 2.1 Tissue culture supernatant from various sources including uncloned primary isolates, molecular clones or pseudo typed virus can be used in the assay. Stocks should be generated as described in consensus protocols for the generation of viral stocks. Viral stocks should be aliquoted in volumes that will allow for one use and then frozen at -70 until testing. Samples should not be subject to repeated freeze thaw cycles. Thaw all samples and bring to room temperature before using in the GHOST (3) assay.
- 2.2 The following GHOST cell lines are available from NIH AIDS Research and Reference Program. *

Chemokine Receptor	Ligand	Predominant Cell Type	Virus that is able to detect (Reported)	
BOB/GPR15			SIV, HIV-2, HIV-1	
Bonzo/STRL33		Activated T-Cells	HIV-1, SIV	
CCR1	Rantes, MIP-1	Monocytes, T-Cells	HIV-2	
CCR2	MCP-1, 3,4	Monocytes, T-Cells	HIV-1 (NSI)	
CCR3	Eotaxin	Eosinophils, Basophiles, Microglia, Dendritic Cells	HIV-1 (NSI)(SI)	
CCR4	TARC	Basophiles, T-Cells		
CXCr4	SDF-1	CD4+ and CD4-	HIV-1 (SI), HIV-2	
CCR5	Rantes	Monocytes, Dendritic Cells	HIV-1 (NSI), HIV-2	
CCR8				
V28/Cx3CR1				
X4/R5				

*You should always include the parental GHOST (3) cell line which contains no co-receptor. Parental cells express low levels of CXCr4 so it is very important to include them in all assays; their use allows for the evaluation of background GFP expression.

3. REAGENTS AND MAINTANCE CELL CULTURE

3.1 Media: Media requirements are different for the parental and co-receptor cell lines. The parental cell line will die if exposed to media containing Puromycin. Media should be prepared fresh and stored for no longer than 1 month. Media should be prepared as follows:

- 3.1.1 Parental:
 - 3.1.1.1 DMEM 90%,
 - 3.1.1.2 FBS 10%.
 - 3.1.1.3 500ug/mL G418,
 - 3.1.1.4 100ug/mL hygromycin
 - 3.1.1.5 Pen/strept (100units/mL/100ug/mL)

- 3.1.2 Co-receptor lines:
 - 3.1.2.1 DMEM 90%,
 - 3.1.2.2 FBS 10%.
 - 3.1.2.3 500ug/mL G418,
 - 3.1.2.4 100ug/mL hygromycin
 - 3.1.2.5 Pen/strept (100units/mL/100ug/mL)
 - 3.1.2.6 1ug/mL Puromycin.

3.2 Cell Culture Procedure

- 3.2.1 Cells should be passaged every 2-4 days.
- 3.2.2 The time between passaging will vary according to the seeded cell density in the new flask, so it is best to observe daily.
- 3.2.3 Do not allow cell monolayers to become more than 80% confluent prior to splitting.
- 3.2.4 Trypsin-EDTA should be used to remove cells from flask.
- 3.2.5 Cells should be used in experiments for no more than 25 passages.
- 3.2.6 When seeding new cells from a frozen stock allow new cells to remain in culture for several passages before using in experiments.

4. SUPPLIES AND EQUIPMENT

- 4.1 Lab Coat
- 4.2 Gloves
- 4.3 Micropipettes capable of delivering appropriate volumes
- 4.4 Disposable pipette tips
- 4.5 Disposable pipettes
- 4.6 Dilution tubes
- 4.7 Sterile Tissue culture plates
- 4.8 Incubator with CO₂
- 4.9 Trypsin
- 4.10 Polybrene
- 4.11 Hemacytometer
- 4.12 Inverted Microscope

- 4.13 Fluorescent Microscope
- 4.14 Camera documentation system (not required)

5. PROCEDURE

5.1 Preparation of cells

- 5.1.1 Media is removed from target cells.
- 5.1.2 Cells are washed once with HBSS or PBS to remove any residual protein.
- 5.1.3 One mL of Trypsin is added to flask and rocked back and forth gently.
- 5.1.4 Flask is placed in 37 C incubator for two to five minutes.
- 5.1.5 Immediately add 9 mLs of appropriate media to flask and pipette to break up clumps.
- 5.1.6 Perform cell count using hemacytometer or automatic cell counter.
- 5.1.7 Dilute cells with appropriate complete media and seed into 12 well tissue culture plates 24 hours before infection. Cell density is 25,000 cells per well. Other tissue culture dishes can be used after adjusting starting cell density.
- 5.1.8 Place plates in 37 C incubator for overnight incubation.
- 5.1.9 After overnight incubation observe plates prior to use for confluency and cell morphology.

5.2 Virus

- 5.2.1 Prepare viral dilutions as needed for individual experiments.
- 5.2.2 Viral stocks can be screened undiluted.
- 5.2.3 Samples should be screened in duplicate using a total volume of 500uL per well.
- 5.2.4 Polybrene should be used when preparing virus for use in experiments to enhance infectivity.
- 5.2.5 GHOST (3) cells are sensitive to DEAE-dextran so this should be avoided.

- 5.2.6 After 24 hours examine cell monolayer and remove media. Add respective complete media to each well in a total volume of 2.0 mL and place in 37 C incubator.
- 5.2.7 Observe daily using a fluorescent microscope for GFP expression.
- 5.2.8 Expression is documented by photography. Infection can also be monitored by FAC sorting and by measuring p24 (HIV) or p27 (SIV).

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Procedure: ACTG Lab Man Ghost Cell Assay

Prepared by: ACTG Laboratory Technologist Committee

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Supersedes Archived Manual: DAIDS Virology Manual for HIV Laboratories, Version January 1997