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

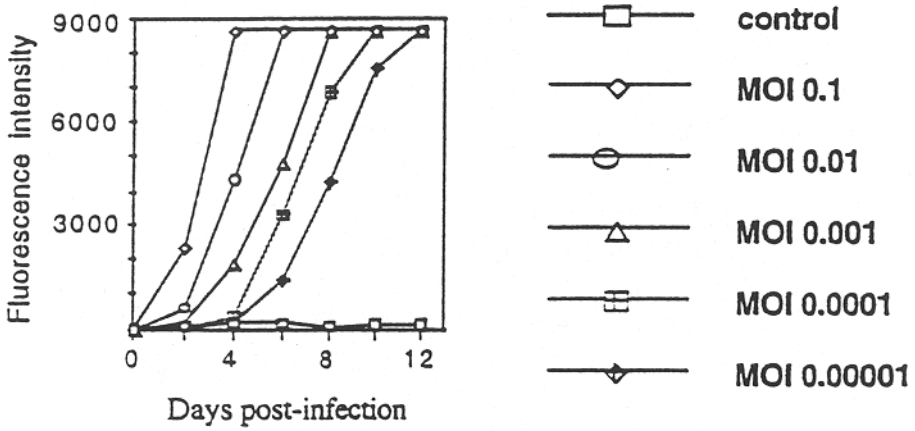

Figure 1: CEM-GFP were infected with HIV-1_{LAI} at different multiplicity of infection (MOI 0.000001 to 0.1) and intensity of fluorescence was measured by fluorimetry using a cytofluorimeter. Note that for each log reduction in inoculum an additional two days was necessary for half maximal detection (5000 arbitrary units).