Chronically infected PM1 HIV-1/Hela Fusion Assay

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1. Aim

This document describes the infection of chronically HIV-1 infected PM1 cells and HeLa CD4-CCR5 (TZM bl) cell lines with the effector vTF7-3 and the reporter vCB21R-lacZ vaccinia viruses as well as the HIV-1 gp120/CD4/CCR5 mediated fusion assay resulting by combining the two cell lines in the same well.

1.1 Definitions and abbreviations

DMEM	Dulbecco's Modified Eagle Medium
FCS	Fetal Calf Serum
CPRG	Chlorophenol red-3-D galactopyranoside
vTF7-3	Vaccinia expressing the T7 RNA polymerase
vCB21R-lacZ	Vaccinia expressing the E.coli lacZ gene under the bacteriophage T7 promoter
CCR5	CC chemokine receptor 5
HIV-1	Human Immunodeficiency Virus 1

1.2 Reagents, vaccinia and cell lines

- DMEM with ultraglutamine (LONZA, Basel, Switzerland) supplemented with 2.5% heat inactivated FCS (LONZA, Basel, Switzerland)
- 2x substrate solutions: 10 mg/ml CPRG (Roche Diagnostics, Mannheim, Germany), 0.12M $Na_2HPO_4x7H_20$, 0.08 M $NaHPO_4x\ H_20$, 0.02 M KCl, 0.002 M $MgSO_4x7\ H_20$ (Sigma Aldrich, Steinheim, Germany)
- Lysing solution: 10% v/v Nonidet P40 in distilled water
- vTF7-3: Recombinant derivative of the vaccinia virus strain WR expressing the bacteriophage T7 RNA polymerase gene under the control of the vaccinia virus p7.5 promoter (catalog number 356) obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID-NIH, USA from Dr. T. Fuerst and Dr B. Moss
- vCB21R-lacZ: Recombinant derivative of the vaccinia virus strain WR expressing the E.coli lacZ gene linked to the bacteriophage T7 RNA polymerase promoter (catalog number 3365) obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID-NIH, USA from Dr. C.C Broder, Dr P.E. Kennedy and Dr. E.A. Berger
- PM1: Clonal derivative of HUT 78 cell lines (catalog number 3038) obtained through the Programme EVA Centre for AIDS Reagents NBSC, UK from Dr P.Lusso
- HeLa CD4-CCR5 TZM-bl: HeLa cell line expressing human CD4 and CCR5 genes (catalog number ARP 5011) obtained through the Programme EVA Centre for AIDS Reagents NBSC, UK from J.C. Kappes and X. Wu

2. Vaccinia infection

- Detach one or more T75 flasks of the HeLa CD4/CCR5 cell line. Use 70% confluent cultures.
- Count and resuspend HeLa and PM1 in DMEM containing 2.5% of fetal calf serum at the final concentration of 10^7 cells/ml in a 50 ml falcon tube.
- Thaw aliquots of the effector (VT7) as well as of the reporter (21R) vaccinia virus.
- Resuspend the vaccinia viruses pipetting up and down with a Gilson pipette
- Sonicate vaccinia (30 sec in ice bath) twice
- Add the sonicated 21R virus to HeLa cells using 10 MOI/cell
- Add the sonicated VT7 virus to PM1 cells using 10 MOI/cell
- Resuspend carefully the infected coltures pipetting up and down the cell suspension
- Rest cells for 2 hours at 37°C in a 5% CO_2 humidified incubator. Every 30 min shake cell suspension. Keep the falcon cap slightly open
- Dilute cell suspension at the final concentration of $0.5x10^6$ cells/ml using DMEM containing 2.5% fetal calf serum
- Rest the infected cultures at 30°C over night in a 5% CO_2 humidified incubator

3. PM1/Hela cell fusion assay

- Centrifuge infected effector (PM1 cells chronically HIV-1 infected superinfected with VT7 vaccinia virus) and reporter (HeLa cells infected with the 21R vaccinia) cultures at 160 g for 10 min
- Discard supernatant count and resuspend PM1 HIV-1 infected cells in fresh medium (DMEM + 2.5% FCS) at the concentration of $0.5\text{-}1x10^6$ cells/ml
- Seed 100 µl of PM1 HIV-1 infected cells infected with the reporter vaccinia virus in a 96 wells flat bottom plate ($5\text{-}1x10^5$ cells/well)
- Incubate 20 min at 37°C
- Add 50 µl of Hela cells infected with the reporter virus (21R vaccinia virus) to each seeded well ($5\text{-}1x10^5$ cells/well)
- Rest the plate for 2 hours at 37°C in a 5% CO_2 humidified incubator
- Lyse cell culture adding 10µl of 10% Nonidet P40 solution (final concentration/well= 0.5% Nonidet P40)
- Resuspend by pipetting the lysed cultures and transfer 50 µl of each well in a fresh 96 wells flat bottom plate
- Add 50 µl of a 2x solution containing CPRG
- Read the plate in a colorimeter using a 570 nm filter