HIV-1 Single Cycle Infection

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[Abstract] The role of a viral or cellular protein on HIV-1 infection can sometimes be difficult to assess in a system where the virus is able to replicate for several cycles. Indeed, some effects are only observable at early time points, and can be masked (or on the contrary, artificially increased) after several rounds of viral replication. Therefore, to clearly know at which step of HIV-1 replication cycle one protein acts, it is important to be able to study one cycle of infection only. This protocol allows rapid and robust quantification of HIV-1 single cycle infection, and can be used to compare mutant viruses, or treatments with different drugs.

Materials and Reagents

1. HeLa $CD4^+$ $CCR5^+$ LTR *lacZ* (also known as P4C5) cells (Verrier *et al.*, 1997).
2. Dulbecco Eagle's Modified Medium (DMEM) (Life Technologies, Gibco®)
3. HIV-1 Δ*env* viral particles, produced by transfection of HEK-293T cells
4. Nonidet P40 (NP40) (Sigma-Aldrich)
   No longer commercially available, but replaced by Igepal CA-630 (Sigma-Aldrich, catalog number: I8896)
5. Phosphate buffer saline (PBS) (Life Technologies, Gibco®)
6. Chlorophenol Red-β-D-Galactopyranoside (CPRG) (F. Hoffmann-La Roche, catalog number: 10884308001)
7. Fetal bovine serum (FBS) (Thermo Fisher Scientific)
8. Penicillin / Streptomycin (PS) (Life Technologies, Gibco®)

Equipment

1. 96-well spectrophotometer
2. Flat-bottom 96-well plates (Corning, Costar®)

Procedure

1. P4C5 cells are HeLa cells, stably expressing CD4 and CCR5. Additionally, they express

the *lacZ* gene under the control of HIV-1 Long Terminal Repeat (LTR) promoter. They are grown like normal HeLa cells, in DMEM, 10% heat-inactivated FBS, 10% PS, and kept at 5% CO_2 and at 37 °C.

2. 8 x 10^4 P4C5 cells were plated in flat-bottom 96-well plates in a final volume of 100 µl of complete medium for 24 h.
3. Cells were infected in triplicate with HIV-1Δ*env*, using 1 or 5 ng of Gag p24 per well. Specifically, 100 µl of virus-containing medium were added to the 100 µl of complete medium already in the well. Viral titers have to be previously determined using a Gag p24 ELISA assay (either home-made or commercial). The use of two different viral inputs is required to make sure that the saturation levels are not reached.
4. At 36 h post-infection, cells were lysed in 80 µl of lysis buffer, then incubated for 1 min at room temperature with 80 µl of lysis buffer, containing CPRG at a final concentration of 3.65 mg/ml. CPRG, the substrate of the β-galactosidase enzyme, allows to monitor the levels of infection. OD (570 nm) was measured every 15 min and normalized against background levels of OD (690 nm).
5. Infection levels - as monitored by percentage of Gag^+ cells by flow cytometry, or quantification of Gag p24 in the culture supernatants by ELISA - are proportionnal to the normalized OD (570 nm). The assay is linear from 0.1 to 3 units of OD (570 nm). Representative results are shown in Figure 3B of Roesch *et al.* (2012).

Recipes

1. Complete medium
   DMEM
   10% heat-inactivated FBS
   10% PS
2. Lysis buffer
   PBS
   0.1% NP40
   5 mM $MgCl_2$

Acknowledgments

This protocol is adapted from Roesch *et al*. (2012).

References

1. Roesch, F., Meziane, O., Kula, A., Nisole, S., Porrot, F., Anderson, I., Mammano, F., Fassati, A., Marcello, A., Benkirane, M. and Schwartz, O. (2012). Hyperthermia stimulates HIV-1 replication. *PLoS Pathog* 8(7): e1002792.
2. Verrier, F. C., Charneau, P., Altmeyer, R., Laurent, S., Borman, A. M. and Girard, M. (1997). Antibodies to several conformation-dependent epitopes of gp120/gp41 inhibit CCR-5-dependent cell-to-cell fusion mediated by the native envelope glycoprotein of a primary macrophage-tropic HIV-1 isolate. *Proc Natl Acad Sci U S A* 94(17): 9326-9331.

