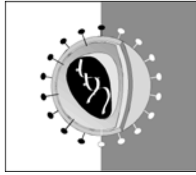

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Propagation of HIV-1_{JR-FL} and HIV-1_{JR-CSF}

Culture Medium: RPMI 1640 with L-glutamine and pen-strep, 80%; fetal bovine serum, 20%

Propagation:

1. Just prior to infection of PBLs, thaw the frozen virus on ice or in cold running water. When thawed, keep the virus on ice until use.

2. Infect PHA-stimulated normal PBLs as follows (use a 15 ml conical centrifuge tube for infection):

   a. Infect 5×10^6-1×10^7 PBLs with the entire vial of thawed virus.

   b. Add polybrene to 10 µg/ml (stock solution should be 1 mg/ml).

   c. Incubate the infected cells for 2 hours at 37°C with occasional shaking.

3. Remove the tube of infected cells from the incubator and centrifuge for 5 minutes at 1500 rpm.

4. Wash the cells twice with RPMI 1640.

5. Resuspend infected cells in 10-20 ml culture medium in a T25 flask. Incubate at 37°C.

6. On day 4 post-infection, centrifuge the cell suspension at 1500 rpm for 5 minutes and discard the cell supernatant. Replace the discarded medium with an equal volume of fresh culture medium, and incubate the cells at 37°C.

7. Harvest virus on day five as follows:

   a. Centrifuge the infected cells at 1500 rpm for 5 minutes. Transfer the supernatant to a new centrifuge tube, and resuspend the pellet in 10-20 ml of culture medium. Incubate the resuspended cells at 37°C.

   b. Centrifuge the supernatant at 3000 rpm for 20 minutes at 4°C. Aliquot and freeze the clarified supernatant.

8. Harvest additional virus on days six and seven as described in step 7. Assay for p24 to confirm the presence on virus.

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.