# Antiviral Agent Testing Using CEM-TART Cells

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The following protocol describes a general procedure for testing antiviral compounds using CEM-TART cells infected with
the proviral HIV-1 mutant MC99IIIBΔTat-Rev. This *rev-tat* defective virus can only reproduce in CEM-TART, a T-cell line that expresses Rev and Tat proteins. A major advantage of using this system for screening antivirals is that the infection is biologically contained; thus, virus recovered from the culture is only replication-competent
in CEM-TART cells.

## I. INFECTION OF CEM-TART CELLS AND PREPARATION OF VIRUS STOCKS

**Reagents:**

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|---|---|
| Cells | CEM-TART cells (Catalog #1944) |
| Culture Medium | RPMI 1640, 80%; heat-inactivated fetal bovine serum, 20%; gentamicin, 1.0 μg/ml |
| Virus | MC99IIIBΔTat-Rev (Catalog #1943) |
| Triton X-100 | 5% stock in water |

**Procedure:**

1. Quickly thaw a vial of CEM-TART and pellet the cells by centrifugation at 500 x g for 10 minutes.

2. Carefully remove the supernatant. Resuspend the pellet in 10 ml of fresh culture medium and transfer the cells to a T25 flask. Incubate the cells at 37°C in a 5% $CO_2$ humidified atmosphere. Note that CEM-TART grows as a suspension culture.

3. After 48 hours, determine the cell number and viability. If the cell number is < 1 x $10^6$ cells/ml, continue the incubation. If the cell number is >1 x $10^6$ cells/ml, transfer the cells to a 15 ml conical tube and centrifuge at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in fresh culture medium at 2 x $10^5$ cells/ml.

4. Repeat step 3 over a period of 7 days to ensure a healthy culture. The viability should be maintained between 90–95%.

5. To initiate the infection, place 2 x $10^6$ CEM-TART cells in a 15 ml conical centrifuge tube and centrifuge for 10 minutes at 500 x g. Discard the supernatant.

6. Quickly thaw a vial of MC99IIIBΔTat-Rev virus in a 37°C water bath.

7. Add 0.5 ml of the virus stock to the pellet and gently resuspend the cells. Incubate for 1 hour in a 37°C water bath.

8. Pellet the cells by centrifugation for 10 minutes at 500 x g. Discard the supernatant and resuspend the cells in 10 ml of fresh culture medium.

9. Repeat step 8, then transfer the cells to a T25 flask. Return the flask to a 37°C, 5% $CO_2$ incubator.

10. After 48 hours, remove 1.0 ml of the suspension culture and clarify the supernatant by

centrifuging at 500 x g for 10 minutes. Transfer 450 µl of the spun supernatant into a tube containing 50 µl of 5% Triton X-100. Freeze the supernanant at -20°C until used for p24 determination.

11. Pellet the cells remaining in the flask by centrifugation, and resuspend them in fresh culture medium at 2 x $10^5$ cells/ml. Continue the incubation. Note that growth and viability will decrease as the infection progresses.

12. Monitor p24 levels in the culture by repeating steps 10 and 11. Replace the culture medium every 2–3 days as it becomes acidic.

13. Prepare virus stocks by harvesting the supernatant between 7–9 days post-infection, when the p24 levels are >1000 pg/ml. The supernatant should be filtered through a 0.45 micron filter (Millipore, Millex HA) and frozen in liquid nitrogen for later use.

**II. TESTING FOR ANTIVIRAL ACTIVITY USING CEM-TART CELLS**

**Reagents:**

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|---|---|
| Antiviral Agent | Prepare at 1X and 2X the desired concentration in culture medium |
| Culture Medium | RPMI 1640, 80%; heat-inactivated fetal bovine serum, 20%; gentamicin, 1.0 µg/ml |
| Cell Line | CEM-TART (Catalog #1943) |
| Virus | MC99IIIBΔTat-Rev (Catalog #1943). Virus stocks with known p24 values are prepared as described above. |

**Procedure:**

1. Place 5 x $10^5$ uninfected CEM-TART cells in 1.0 ml of medium into each well of a 6-well plate. Use as many plates as needed for the desired number of antiviral agents and/or concentrations being tested.

2. To all but the control wells, add 1 ml of culture medium containing the 2X antiviral agent. Incubate the plate at 37°C in a 5% $CO_2$ humidified atmosphere. The control wells should receive only culture medium and virus throughout the experiment. Note that the antiviral agent is now at a concentration of 1X in 2.0 ml.

3. After one hour, add 1 ml of MC99IIIBΔTat-Rev virus at 3.0 ng p24/ml to each well. The virus should be diluted to 3.0 ng p24/ml just prior to use and kept on ice until added to the wells. Note that the antiviral compound is now at a concentration of 0.67X in 3.0 ml.

4. Continue the incubation. After one hour, add 1.0 ml of 2X antiviral compound to adjust the antiviral concentration back to 1X in 4.0 ml.

5. Incubate the plate for 48 hours.

6. Transfer the cells in each well to 15 ml conical centrifuge tubes. Centrifuge the cells for 10 minutes at 500 x g, remove the supernatant, and resuspend the cells in 10 ml of culture medium.

7. Pellet the cells by centrifugation again, but resuspend the cells in only 1 ml of culture medium. Perform a cell count. Dependent on these results, add $5x10^5$ cells and sufficient culture medium containing 1X antiviral agent to each well to bring the volume to 2 ml.

8. At five days post-infection, repeat steps 6 and 7.

9. On day 7 (and at subsequent time points depending on the study), collect the supernatant from each well and assay for soluble p24. Any effect of the antiviral agent may be determined by comparison to the control samples.