Propagation of Virus-infected H9 Cells and Collection of Virus Supernatant: H9/HIV-2MVP-15132 and H9/HIV-2MVP-11971

Culture Medium: RPMI 1640 supplemented with 10% fetal bovine serum, 30 µM 2-mercaptoethanol, 600 mg/L L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin

Propagation:

1. Wash out DMSO from infected cells as follows: Partially thaw the frozen ampule quickly in a 37°C water bath. Transfer vial containing frozen and liquid stock into a 50 ml centrifuge tube containing 40 ml culture medium. Gently shake the centrifuge tube, then centrifuge at 400 x g.

2. Remove all of the supernanant and resuspend the pelleted cells in 5 ml of fresh culture medium. Transfer the cells to a 50 ml culture flask. Incubate overnight at 37°C in a 2-5% CO_2 atmosphere.

3. The culture medium should be yellow after one day in culture. If it is yellow, add 5-10 ml culture medium. If the medium is not yellow, wait one day; if it is still not yellow (this will rarely occur), supplement the culture with fresh H9 cells, then add 5-10 ml culture medium after yellowing occurs.

4. Two to three days after the addition of medium, the culture should again turn yellow. Split the cells into three 50 ml culture flasks when this occurs, and add 5-10 ml of culture medium to each flask.

5. Once the medium in the three 50 ml culture flasks has turned yellow, combine the contents of these flasks into one 250 ml culture flask and add medium to turn the culture medium pink (total flask volume will be about 40-50 ml). Add 5 ml of culture medium to the cells remaining in the 3 small flasks and incubate; enough cells will remain in the flasks to start the culture again, if necessary.

6. The medium in the 250 ml culture should turn yellow again within a week. Add fresh medium to the culture as needed; addition of fresh H9 cells is not necessary.

7. Virus can be harvested at a titer of $10^{4.5}$ $TCID_{50}$/ml between 4 and 8 weeks of culture. Virus titer decreases after 8 weeks of culture. After 8 weeks, a new culture should be started by infecting 40 ml of H9 cells (1 x 10^6 cells/ml) with 3 ml of infected medium.