**Care and feeding of 293T and 293T-CEP4SIVtat cells**

**Directions for 25 cm² cell culture flasks:**

Culture 293T cells in complete DMEM and 293T-CEP4SIVtat cells in complete DMEM plus hygromycin.

Need:

-Phosphate buffered saline (PBS)
-Non-enzymatic cell dissociation solution (Versene 0.2 g/L EDTA4Na in phosphate-buffered saline)
-Complete Dulbeccos minimal essential medium (DMEM) with 10% fetal calf serum (FCS) and 1X penicillin-streptomycin
- Complete Dulbeccos minimal essential medium (DMEM) with 10% fetal calf serum (FCS) and 1X penicillin-streptomycin AND HYGROMYCIN B (250 μg/ml) (for 293T-CEP4SIVtat cells only)
-Cell culture flasks
-15 ml sterile centrifuge tubes
-Pipets
-Micropipeter

1. Thaw one tube of 293T or 293T-CEP4SIVtat cells quickly in a 37°C water bath.
2. Upon thawing immediately place cells in a 25 $cm^2$ cell culture flask with 10 ml of pre-warmed complete DMEM without hygromycin
3. Incubate at 37°C, 5% $CO_2$ in a cell culture incubator
4. After cells have formed a confluent monolayer (about 7 days), remove media and wash once with PBS. Tat producing cells are microscopically polymorphic and grow quickly once established.
5. Add 2 ml non-enzymatic cell dissociation solution (versene, 0.2 g/L EDTA4Na in phosphate-buffered saline) and incubate at 37°C for 5 min. The Tat-producing cells seem to be sensitive to trypsin and are easily lysed, therefore non-enzymatic dissociation is recommended.
6. You will be able to see the cells detaching from the flask but a few gentle taps can assist the process for all of the cells
7. When you see that most of the cells have detached from the surface of the flasks, pipet the dissociation solution up and down a few times over the surface to dislodge any cells from the plastic
8. Place the cells in a 15 ml tube and add 10ml complete DMEM
9. Centrifuge cells at 500 x g for 10 min
10. Remove media and discard.
    a. Gently tap the tube to break up cell clumps and dislodge the pellet. This usually takes about 10 taps.
11. Add 5 ml of complete DMEM to cells and pipet up and down a few times to further break up cells.
12. To pass cells into another 25 $cm^2$ flask, use 1 ml per new flask and add ~7 ml complete DMEM (for 293T cells) or ~7 ml complete DMEM plus hygromycin (293TCEP-SIVtat cells). To pass to larger flasks, scale up volumes accordingly.
13. Incubate at 37°C in 5% CO2
14. Using this protocol your cells will be ready to split again in about 3-4 days. If you want to slow them down, pass fewer cells to the new flasks. You can split these cells 1:20 and still have a confluent flask within a week.