



CELL LINE BANKING IN THE CSC

1. Thaw the cell recovery medium, Invitrogen Cat# 12648010, along with the media and the trypsin, Fisher Cat # MT25053CI
2. Swirl (to catch all dead cells that are not adhered to the surface) and aspirate off all medium using 2 mL pipette connected to a vacuum hose
3. Add 0.25% Trypsin-EDTA, (3 mL for T-75s, Fisher Cat # 10-126-37 and 5 mL for T-175s, Fisher Cat# 10-126-61)
4. Incubate flask in 37 deg incubator for 5-7 minutes
5. Check under microscope for detached cells
6. If not detached properly, give slight hand taps to the side of flask. Repeat step 4
7. If cells still not detached, incubate flask in the 37 deg incubator for another 1-2 minutes. Repeat steps 4, 5
8. Add media (refer to Cell Bank Log for appropriate media details) to neutralize trypsin effects (3 mL minimum for T-75s and 5 mL minimum for T-175s)
9. Wash the flask surface at least 10 times with the medium in the flask using a pipette
10. Pool the cells from all the flasks together into a 50 mL centrifuge tube
11. Count cells in duplicate using Vi-Cell XR (refer to Vi-Cell Protocol by NP for details).
12. If continuing the cell line, add 1 mL of cells to a new medium containing flask (Media - 15 mL for T-75s and 25 mL for T-175s)
13. Label the new flask with details
 - a) Cell Type b) Passage Number c) Date d) Initial of person doing the steps e) Split Ratio f) Medium Type including g) % of FBS
14. Adjust volume to have 46 million cells (1 million per vial, 45 vials, 5 reserve + 40 working + 1 mL extra to adjust for pipetting)
15. Centrifuge for 5 minutes at 2000 rpm at 25 deg C
16. Carefully aspirate off all the medium (supernatant) leaving the pellet of cells intact
17. Resuspend cells in 30 mL sterile PBS, Fisher Cat# 100-100-23, 1x and re-centrifuge at above parameters (step 14). This is to wash off any unwanted cell debris
18. Repeat Step 15 to remove all PBS (supernatant)
19. Resuspend cells in appropriate amount of cell recovery medium, (x mL, x = number of millions of cells in tube)
20. Aliquot in labeled Nunc cryotubes, Fisher Cat# 12565171N, 1 mL each and store in propanol containers at -80 deg C overnight
21. Transfer the cryotubes from -80 deg C to liquid nitrogen storage the following day
22. Please note the **EXACT** location of cells (rack #, ex: 1A or 1B, and location#, ex 1A has the HeLas, locations 1-5 have R01-R05 and 6-45 have W01-W40, etc and add all details to cell banking log. For further details, please refer to Labeling Guidelines Protocol by NP or contact Sam Bettis, Nicolette Pavlovics or Swadeep Pillarisetti for further assistance