Procedure for Passaging Cells in T-25 or T-75 Flask

1.      Warm media and trypsin in 37C water bath.

2.      Check cells in T flask under microscope to confirm that the cells are 90%-100% confluent.

3.      Clean hood with ethanol.

4.      Sterilize all materials, bottles, etc. which are loaded into the hood. Spray hands with ethanol. Jars of liquid need to be sprayed with ethanol. Sterile pipets may be placed in the hood directly. Automatic pipettors should enter the hood WITHOUT sterilization.

5.      Spray hands with ethanol. Remove T flasks from the incubator and quickly place in hood. (As general guidance, do not spray flasks with ethanol.)

6.      Attach an aspirating pipet to the tube attached to vacuum. Turn on vacuum system by opening vacuum valve in hood.

7.      Using the aspirator, empty liquid media covering cells. Be careful to not touch the pipet to anything outside of the T flask.

8.      Add 10 mL of PBS to T flask. Lightly swish PBS on base of T flask. Aspirate PBS from T flask.

9.      Add 4 mL trypsin to T-75 flask. Lightly swish trypsin.

10.  Place flask in incubator for 5 min, or until detached.

11.  Remove cells from incubator. Tap side of flask on hard surface or your hand. Repeat several times. Visually check to ensure lumps of cells are dispersed.

12.  Check cells under microscope to confirm that cells are detached from the surface.

13.  Add 6 mL of media to dilute trypsin. (Note: The liquid suspension now contains the cells.) Carefully resuspend cells (see Tips for Sterile Technique and Cell Manipulation).

14.  Pipet mixture out of flask and put in 15 mL centrifuge tube. Label tube. Using a microscope, confirm that there are no/few cells left in your flask.

15.  Centrifuge cells (remember counter-weight in centrifuge) for 4 min at 650 g. [Time and RPM good for HDF and CHO cells; other cell lines may require different conditions.]

16.  While centrifuge is spinning, pipet appropriate volume of fresh media (see Table 1, column 2) into new T flasks. Prepare 1 or 2 flasks, as needed. Label with name, date, cell type, passage number and passage dilution.

17.  After centrifugation, aspirate supernatant. Cell pellet should remain at base of tube.

18.  Resuspend cells in 6 mL, 8 mL, 10 mL or 12 mL of media, as appropriate (see Table 1, column 3). See Tips for Sterile Technique and Cell Manipulation for more detailed instructions.

19.  Aliquot appropriate volume of cell suspension into freshly prepared T flasks with media (see Table 1, column 4).

20.  Swish media and cells to mix. Place flasks in incubator.

21.  Turn off aspiration.

22.  Dispose of liquid and solid biohazards wastes properly.

23.  Clean hood with ethanol. Spray ethanol liberally over surfaces and wipe clean with kimwipe.

Table 1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dilution | �Media in fresh T flask (step 16) | Resuspension Media (step 18) | Cell suspension aliquots (step 19) | Total volume in flask (step 20) |
| 1:2 | 6 mL | 8 mL | 4 mL | 10 mL |
| 1:4 | 8 mL | 8 mL | 2 mL | 10 mL |
| 1:8 | 9 mL | 8 mL | 1 mL | 10 mL |
| 1:10 | 9 mL | 10 mL | 1 mL | 10 mL |
| 1:12 | 9.5 mL or 9 mL | 6 mL or 12 mL | 0.5 mL or 1 mL | 10 mL |
| 1:16 | 9.5 mL | 8 mL | 0.5 mL | 10 mL |

Table 2

|  |  |
| --- | --- |
| Dilution | Approximate time  until next passage |
| 1:2 | 1-2 d |
| 1:4 | 3-5 d |
| 1:8 | 5-7 d |
| 1:10 | 6-8 d |
| 1:12 | 7-9 d |
| 1:16 | 8-10 d |

NOTES:

Brain Endothelial Cells when plated at a 1:10 mL dilution reaches confluency in 72 hours (3 days) in a T – 25 Flask

* As a result, will switch cells to a T – 75 Flask at a 1:10 mL dilution to assess how the growth kinetics change given the increase in flask volume (25 cm^2 to 75 cm^2).