Materials and Equipment

Materials

- Cells to be subcultured
- 50 ml tube of cells
- DPBS
- Flask (variable)
- FBS (or other enzyme inhibitor)
- Cell culture media
- 2ml glass pipet

Equipment

- Centrifuge
- Biosafety Cabinet

Procedure

1.	NOTES: Except centrifugation, entire procedure must be done in the Biosafety Cabinet using aseptic techniques. Cells should never be left without a fluid layer. Use 2 ml glass pipet or similar to aspirate all media from flask. Cells must never remain in trypsin for longer than 5 minutes.
2.	Properly pre-label 50 ml conical tubes with cell line designation.
3.	Remove flask with cells and flask from incubator.
4.	Observe cells under microscope to make sure that they are healthy and have no signs of contamination. In most cases (unless specified) cells should be in "log phase" of growth (at the edge of confluency). Most cells should not exceed 80% confluency
5.	Aspirate media
6.	Add warm PBS, gently rock flask/plate to wash, aspirate PBS
7.	Slowly add trypsin to the cells using the volumes indicated in the table below:

Working Volumes for Trypsinization

Flask Type	Flask Size	Vol. of Trypsin
Round Dishes	35 mm	0.2-0.5 ml
	60mm	0.5-0.9 ml
	100 mm	2.0 ml
	150 mm	3.0 ml
Multi-well Plates	6-well	0.2-0.3 ml
	12-well	0.1-0.2 ml
	24-well	0.08-0.1 ml
	48-well	0.05 – 0.08 ml
	96-well	0.01 – 0.02 ml
Flasks	T-25	1.0 ml
	T-75	5.0 ml
	T-175	8.0 ml
	T-300	15 ml

8.	Place flask with trypsin back into incubator.
9.	After no more than 5 minutes, use microscope to confirm that cells have dislodged from flask surface.
10.	Neutralize Trypsin by addition of 10% of FBS or enzyme inhibitor. Remove cells in trypsin to conical tube
11.	Pipet gently down lower surface to dislodge remaining cells and to stop enzymatic reaction.

12.	Transfer cells to the pre-labeled conical tube.
13.	Wash cell culture flasks/plates with equal volume (to trypsin used) PBS and add to prelabeled conical tube.
14.	Centrifuge at 400g for 5 min at 4°C.
15.	Carefully aspirate supernatant. Be careful not to disrupt the cell pellet when removing the supernatant.
16.	Resuspend the cells in 1 ml Media or FBS.
17.	Count Cells (See How To Count Fresh Cells)