Splitting Cells

For a 150 mm dish of 3T3 cells:

All reagents should be warmed to 37˚ prior to use.

1. Aspirate medium from cells using sterile pipette or flamed needle
2. Rinse plate with several ml HBSS and aspirate the wash
3. Add 4 ml HBSS plus 2 ml trypsin/EDTA to the plate.
4. Place plate in tissue culture incubator and incubate 5 to 10 min
5. Label new dishes and add 25 ml medium while waiting.
6. Remove plate from incubator and tap sharply to release cells from dish. Using a sterile pipette, triturate the HBSS/trysin solution to release any remaining cells.
7. Transfer cells to new dishes at desired concentrations

Note: This protocol works well for 3T3 and KB cells which come of fthe plate easily. A431 cells require more trypsin (4 ml trypsin/EDTA plus 2 ml HBSS) and more time to be adequately released from the plates. CHO cells are trypsin-sensitive and should be trypsinized with ten-fold diluted trypsin (ie. 0.6 ml trypsin plus 5.4 ml HBSS).

Dilution Chart:

Shows the number of plates (at a 1:1 split) you get out of a dish of a particular diameter.

If you have -→ You get this many	**D150**	**D100**	**D60**
D150	1	-	-
D100	2.25	1	-
D60	6.25	2.77	1
D35 (6 well dish)	18	8.16	2.94
D22 (12 well dish)	46	20.8	7.5
D15 (24 well dish)	100		