PROTOCOL FOR FASC ANALYSIS OF Cell Cycle using BrdU and PI

Labeling of Cells with BrdU:

Add BrdU (Sigma) at a final concentration of 10 uM to approximately 1 x 10^6 cells, and incubate under the appropriate growth conditions for 15 to 60 minutes to pulse label the cells. Times may vary depending on the cell line.

Collecting the Cells:

1. Scrape or trypsinize the cells if using adherent cells. If you are interested in analyzing cell death, collect cells floating in the media as well as the adherent population.

2. Pellet the cells at 800 to 1000 rpm for 5 to 10 minutes and remove the supernatant.

3. Wash the pellet in 5 ml of 1X cold PBS and remove the supernatant.

4. Resuspend the pellet gently in 100 ul of cold PBS and keep the cells on ice.

5. Fix the cells by dropping them slowly into 5 ml of ice cold Ethanol while maintaining a Gentle vortex.

6. Place the cells at 4^oC for at least 30 minutes or at 4^oC overnight, which is the preferred method for optimal fixation.

Processing the Cells:

1. Spin down the Ethanol fixed cells and remove the supernatant leaving a small amount of liquid in the bottom of the tube (approximately 50 - 100 ul).

2. Gently vortex the cells and slowly add 1 ml of 2N HCl/Triton x-100 to denature the DNA.

3. Incubate at room temperature for 30 minutes.

4. Spin down the cells and remove the supernatant.

5. Resuspend the cells in 1 ml of 0.1 M $Na_2B_4O_7$, pH 8.5 to neutralize the sample.

6. Spin down the cells and remove the supernatant.

7. For each sample, make up a master mix that includes the following:
   50 ul of 0.5% Tween 20/1% BSA/PBS
   20 ul of anti-BrdU-FITC (Becton Dickinson)
   5 ul of RNAse (10 mg/ml)

8. Add 75 ul of the master mix to each sample and incubate at room temperature for at least 30 minutes. Can store at 4^{o}C overnight, which is the preferred method for optimal staining.

9. Spin down the cells and remove the supernatant.

10. Resuspend the cells in 1 ml of PBS containing 5 ug/ml PI (Sigma) and store in the dark.

11. Analyze all samples by flow cytometry.

Solutions:

2 N HCl/0.5% Triton = 83.33 ml conc. HCl
2.5 ml of Triton X-100
bring up to 500 ml in dH_2O

0.1 M $Na_2B_4O_7$ = 19.07 g sodium borate
bring up to 500 ml in dH_2O
pH to 8.5 with HCl