Protocols

Cell Cycle Analysis using Propidium Iodide (PI)

1. Harvest cells and prepare single cell suspension in buffer (e.g. PBS + 2% FBS; PBS + 0.1% BSA)
2. Wash and spin cells at 300 x g for 5 minutes 2 times. Resuspend at 3-6 x 10^6 cells/ml.
3. Aliquot 500µl cells in a 15 ml polypropylene, V-bottomed tube and add 5 ml cold 70% ethanol drop-wise while gently vortexing. *If cells are not vortexed on addition to the ethanol, they will be fixed to each other in clumps.* Fix cells for at least 1 hour at 4°C. (Cells may be stored in 70 % ethanol at -20°C for several weeks prior to PI staining and flow cytometric analysis).
5. Wash cells 2 times in PBS as described above. (It may be necessary to centrifuge cells at a slightly higher "g" to pellet after ethanol fixation.)
6. Add 500µl of PI staining buffer and incubate 3 hr at 4°C.
7. Store samples at 4°C until analyzed by flow cytometry.

PI Stock, 1mg/ml

Dissolve 1mg Propidium Iodide (Sigma # P 4170) in 1ml distilled water. Store at 4ºC in the dark.

RNase A (DNase free) stock solution, 2mg/ml

Dissolve 2mg RNase A (sigma # R 6513) in 1ml distilled water. Store at 4ºC.

PI staining buffer

(1X PBS, 2%FBS, 50µg/ml PI, 200µg/ml RNase A, .1% Igepal)

To 9.5ml 1XPBS w/ 2%FBS add;

1.) 1.) 500µl PI stock (1mg/ml)
2.) 2.) 10µl RNase A stock
3.) 3.) 10µl Igepal (Sigma # CA-630 *substitute for NP40*)