Protocol: Cell Cycle/Whole Cell

Reagents:

1.) Ice cold 100% Ethanol
2.) Propidium Iodide (PI) staining solution: To 10ml of PBS, add 500µl of a 1mg/ml solution of PI, 2mg of RNase A, and 100µl of 10% Triton-X solution (final concentrations: 50µg/ml PI, 0.1% Triton-X).

Procedure:

Ethanol fixation:

1. Wash 10^6 cells twice in 2ml PBS. (Centrifuge at ≅200 x g for 5 minutes, decant, vortex, repeat).
2. Thoroughly resuspend cells in 200µl PBS-it is essential that cells be in single cell suspension prior to fixation with ethanol.
3. Add 800ul of ice-cold 100% ethanol drop-wise while gentle vortexing.
4. Keep cells in fixative ≥ 2 hours.

Note: *Cells suspended in 80% ethanol can be stored at -20°C for several weeks.*

PI staining: (This step can cause lots of cell loss)

5. Add 1ml of PBS to cells.
6. Spin at 800g for 5min
7. Aspirate and repeat wash with 2ml PBS
8. Resuspend cell pellet in 500µl PI staining solution; vortex and incubate for 20minutes at 37ºC.
9. Cover with foil and store at 4ºC until ready to analyze on flow cytometer.