Protocol: Cell Cycle Staining-DAPI

Cell Prep and Staining

1. Harvest cells - wash 2X in PBS to get rid of serum proteins. 1200rpm, 5 min.
2. Resuspend pellet (up to $3x10^6$ cells) in 1.2 ml PBS (Ca and Mg free).
3. Add 3.0ml ice cold 95% EtOH dropwise while vortexing. This crosslinks proteins.
4. Fix in this final 70% EtoH solution for at least 30 min. The cells can remain in this solution for up to one week.
5. Dilute EtOH/cell suspension with 12mL PBS (for a total volume of 15mL). Spin at 2000-2200 rpm for 10 min. Cells are much harder to pellet in EtOH. If EtOH is not diluted and the increased rate is not used significant cell loss will be noticed.
6. Decant, and wash again in 15mL PBS; continue spinning at 2000-2200 rpm for 10 min.
7. Count cells.
8. Resuspend in 0.5-2.0ml DAPI stain solution (final concentration of $1x10^6$ cells/ml) and incubate for 30 min. at 4°C or on ice.

DAPI Stain Solution

0.1% (final concentration) TritonX 100 in 10ml PBS
add 100ul of 1mg/ml DAPI to the 10ml TritonX solution