Cell Cycle Preparation

Fixing of cells

After treatment (i.e., drug treatment, incubation with agonist, etc.), pour off medium and collect cells using trypsin in 15 ml tubes.
Spin down cells, discard supernatant.
Wash cells with cold 5 ml PBS. (Vortex GENTLY)
Spin down cells, discard supernatant.
After discarding supernatant, vortex the cell pellet GENTLY (with the little PBS left behind). This is to avoid clumping of cells.
Add 0.5 ml of cold PBS and 5 ml of 70% ethanol.
The fixed cells can stay at 4C in the dark for up to 4 weeks.

PI staining

Spin down the cells, discard ethanol.
Wash with 5 ml of cold PBS (vortex briefly)
Spin down, discard PBS
Vortex the cell pellet in the little amount of PBS left behind to avoid clumping of cells.
Add 500 ul RNase and 500 ul PI
Stain in dark for 45 minutes.
Transfer cells through the meshed blue capped falcon tubes (# 352235). This is again done to avoid clumps.
Ready for FACS analysis.
KEEP SAMPLES PROTECTED FROM LIGHT AND ON ICE FOR TRANSPORT TO FACS LAB
[RECOMMEND READING THE CELLS ON CYTOMETER WITHIN 24 HRS.]

Solutions

PI solution

0.1 mg/ml PI in 0.6% Triton-X in PBS. This can stay at room temp for a few days. Keep in dark. Wrap Al foil or make in dark brown bottle.

For 10 ml: 1 mg of PI + 60 ul of Triton-X and make up volume to 10 ml
PI (Sigma) P4170

RNase solution

Ribonuclease A (# R-5125, SIGMA)
Make 2 mg/ml in milli-Q water. Use 500 ul for each tube.