Propidium Iodide Staining for DNA Content

Propidium Iodide (PI) is a dye that can be used in FACS to analyze DNA content of lymphocyte populations. It binds intercalated DNA allowing the state of cell cycle to be analyzed. PI fluoresces brightly in the FL2 range; so, FITC-conjugated cell surface antibodies are best when staining secondary targets.

If experimental conditions do not call for surface marker expression, skip Step 2.

1. Stimulate cell samples at various time points.
2. Wash and stain for cell surface markers using the current protocol for FACS staining.
3. After 30 minutes, wash cells in 2% FBS in PBS followed by another wash in PBS, and then centrifuge and decant the supernatant.
4. Resuspend cells in 300ul of 50% FBS in PBS.
5. Add 900ul of ice-cold 70% ethanol while vortexing and incubate on ice for 2 hours.
6. After 2hrs wash cells twice in 1x PBS and then resuspend in 900ul PBS containing 6.25 mM $MgSO_4$ and 1mM $CaCL_2$ and incubate for 15 min at room temperature.
7. After incubation, add 20ul of 10mg/mL Rnase A (Gibco BRL, Grand Island N.Y.) incubate at 37° C for 15 minutes.
8. Add 100ul of 500ug/ml propidium iodide (Sigma) and analyze by FACS.
    a. Alternatively, cells can be kept at 4° C overnight and then analyzed by FACS.