Propidium Iodide Staining Protocol

Propidium Iodide Stock (Sigma): 1 mg/ml in PBS
Very hazardous, keep protected from light, use gloves and eye protection

Hypotonic Buffer (1X, in ddH_2O):
0.1% Na Citrate
0.1% Triton X-100

Wash Buffer (AKA FACS Buffer):
Cold PBS with 5% FBS (junk) and 0.01 M NaN_3

1. Count cells (1-5 x 10^5 cells for optimal staining but can use much less).
2. Wash cells with cold wash buffer.
3. Spin down (5 min, 2000rpm).
4. Remove supernatant, save pellet.
5. During spin, prepare PI solution – 10 µl PI stock for every ml of hypotonic buffer.
6. Add 150-500 µl PI in hypotonic buffer to each sample. 200 µl is usually good for 250,000 cells, but this can be varied.
7. Vortex briefly.
8. Incubate in refrigerator at least several hours (or up to 48 hr) before running. If running samples sooner, incubate at room temperature 30-60 min. and run within a couple hours. All incubations should be in the dark.
9. When finished, inactivate PI solution with bleach. FACS machine must be cleaned with Windex – 1 min. with Windex on probe with lever in running position, then 30 sec with lever open (position where you'd switch tubes). Follow this with normal FACS cleaning procedure – 5 min FACS Rinse, 5 min water.

For apoptotic populations, collect on log FL-2 and enumerate sub-G_0 DNA content. For cell cycle analysis, collect on lin FL-2 and enumerate G_0/G_1, S, and G_2/M.

On 5th floor FACS machine, cells often have to be run on low to prevent a large sub-G_0 peak from cell shearing.

Adapted from Nicoletti, et. al., J. Imm. Meth. 139:271-279, 1991.