

DNA staining for cell cycle (adapted from Zbigniew Darynkiewicz's method):

Materials: 70% ethanol, cells to be stained, phosphate buffered saline (PBS), and Propidium iodide/Triton X-100 staining solution with RNase A for ethanol fixed cells (to 10 ml of 0.1% (v/v) Triton X-100 in PBS, add 2 mg DNase-free RNase A and 200 μ l of 1 mg/ml PI)

1. Prepare the fixative by filling 12 x 75 mm tubes with 4.5 ml of 70% ethanol, keep tubes on ice.
2. Wash 10^6 cells in 5 ml of PBS.
3. Centrifuge cells at 200 x g.
4. Aspirate off the supernatant and re-suspend the cells in 0.5 ml PBS. It is very important to disperse the cells into a single cell suspension, if you fix the cells as aggregates, you will be unable to disperse them into a single cell suspension for flow cytometry.
5. Transfer the cell suspension into the tubes containing 70% ethanol. Keep the cells in fixative >2 hr. If need be, cells suspended in 70% ethanol can be stored at -20°C for years!
6. Centrifuge the ethanol-suspended cells at 200 x g. Decant off the ethanol.
7. Re-suspend the cells in 5 ml PBS, wait 60 seconds and centrifuge the cells at 5 minutes at 200 x g.
8. Re-suspend the cells in 1 ml PI/Triton X-100 staining solution with RNase A. Keep either at 15 minutes at 37°C or 30 minutes at room temperature. Measure cell cycle on the flow cytometer.

Most DNA binding dyes can be used in conjunction with other cell markers to define the cell cycle status of subsets of mixed cell populations. Stain your cells for surface and intracellular markers as usual and then fix in paraformaldehyde. Add the DNA binding dye to the cells in 1 ml Triton X-100 staining solution. Incubate for 30 min at RT in the dark before running the samples. Please see the flow core staff for a more detailed protocol.

In the flow core, we have successfully obtained reproducible cell cycle data with the DNA probes listed in Table 1:

TABLE 1: Some dyes suitable for DNA staining.			
DNA Probe	Excitation λ (nm)	Emission λ (nm)	Manufacturer
DAPI	345	455	Invitrogen
Hoechst 33342	350	461	Invitrogen
DyeCycle Violet*	369	437	Invitrogen
Propidium Iodide (PI)	536	617	Invitrogen
7-AAD	546	647	Beckman Coulter
DRAQ5	647	681/697	eBioscience
*DyeCycle Violet will stain live cells but be pumped out by the ABCG2 membrane efflux pump.			