Ethanol-fixation of Samples for Long-term Storage and Subsequent DNA Staining

I. Materials

70% Ethanol at – 20^{o}C

DNA Staining Buffer:

Sodium citrate	0.25g
Triton–x 100	0.75ml
Propidium iodide	0.025g
Ribonuclease A	0.005g
Distilled water	250 ml

II. Procedure

1. Place 1x10^{6} cells from each sample into a polypropylene tube and centrifuge at 250 x g for 5 min.

2. Remove the supernatant as completely as possible without disturbing the pellet and add 1 ml of –20^{o}C 70% EtOH dropwise to the cell pellet while vortexing.

3. Keep cells at -20^{o}C until the day of DNA staining (cells can be stored for several weeks at -20^{o}C).

4. On the day of DNA staining, take samples out of the freezer and spin them down by centrifugation at 250 x g for 5 min. Remove the supernatant as completely as possible without disturbing the cell pellet.

5. Add 1 ml of DNA staining buffer to the cell pellet and vortex gently and briefly. Keep cells for 15 min in the staining solution before acquisition on the flow cytometer.

Commercial sources:

Sodium citrate	Cat# C7254	Sigma, St. Louis, MO
Triton-x 100	Cat# x-100	"
Ribonuclease A	Cat# R4875	"
Propidium iodide	Cat# 537059	Calbiochem, San Diego, CA