DNA STAINING FOR CELL CYCLE ANALYSIS

I. SAMPLE - one or more of the following preparations

A. Suspension of single cells from tissue (e.g., lymph node, spleen, bone marrow, placental cells)

B. Tissue culture cells, including adherent cells detached from support

C. Ficoll-hypaque separated mononuclear cells

II. REAGENT - hypotonic staining buffer for DNA

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

We have kept this solution in a tightly-sealed bottle protected from light for several months without apparent loss of staining activity

III. STAINING

1. Place 1x10^6 cells into each tube.

2. Spin down samples and remove supernatant as completely as possible without disturbing the pellet.

3. Add 0.5 mL of the hypotonic DNA staining buffer to the pellet and mix well.

4. Keep samples at 4°C protected from light for 15 min or for a maximum of 1 hr before acquisition on the flow cytometer. Note: Prolonged exposure to the hypotonic buffer can lead to an increase of debris in the sample and fragile cell types may only tolerate short exposure to the staining solution.

Reference: Krishan A: Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *J. Cell Biol.* 66:188-193, 1975.

Possible commercial sources:

Sodium citrate	Cat# C7254 Sigma-Aldrich, St. Louis, MO
Triton-x 100	Cat# x100 Sigma-Aldrich, St. Louis, MO
Ribonuclease A	Cat# R4875 Sigma-Aldrich, St. Louis, MO
Propidium iodide	Cat# 537059 EMD Millipore, MA