

**Propidium Iodide Staining for Sub-G₁ Analysis:
Hypotonic Lysis Method**

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This method will result in free nuclei, stained with propidium iodide for measurement of relative DNA content. Cells undergoing apoptosis will lose part of their DNA (due to the DNA fragmentation in later apoptosis). Those cells may be detected as a “sub-G₁” population.

Reagents:

Sodium Citrate
Triton X
RNAse (Type I-A)
Propidium Iodide (PI)
Deionized (DI) H₂O
PBS (Ca⁺⁺/MG⁺⁺ free)

PI-Hypotonic Lysis Buffer: (in DI H₂O)

0.1% Sodium Citrate
0.1% Triton X
100 µg/ml RNAse
50 µg/ml PI

Procedure:

Harvesting, washing and counting methods will vary with cell type, origin, and treatment. Buffer is optimized for 1 x 10⁶ cells per sample. Using more or less may require adjustments.

- 1) Aliquot 1 x 10⁶ cells to 12 x 75 mm tube.
- 2) Wash once with cold PBS. (optional)
- 3) Pellet, remove supernatant.
- 4) To pellet: Add 500 µl PI-Hypotonic Lysis Buffer.
- 5) Agitate pellet gently.
- 6) Analyze by flow cytometry after a minimum 20 minute incubation; within 2 hours of hypotonic treatment.

Changes in cellularity may result in artifactual shifts in DNA peaks, as the available dye per cell ratio varies. We recommend use of an internal DNA standard (trout erythrocyte nuclei) to correct for this, available from BioSure (Grass Valley, CA . . . see flow lab personnel for more information).

This protocol is a condensation of efforts from Dr. Arne Kolstad and Missy Tuck (3-4428), and M. KuKuruga (flow core).