DNA Content Protocol

Reagents

		PI Master Mix	
Propidium Iodide (PI) 1mg/mL stock:	**$[PI]_F$ =** 40ug/mL	PI	40uL
RNase (DNase-free) 10mg/mL stock:	**$[RNase]_F$** = 100ug/mL	RNase	10uL
PBS, Ca^{++}, Mg^{++}- free		PBS	950uL
70% Ethanol			1,000uL

Procedure

1. Wash 1-2X106 single cells (perform an initial cell count with hemocytometer) in cold PBS.

2. Re-suspend cells in 200uL cold PBS and vortex.

3. In a 15mL conical centrifuge tube (or 12X75mm round-bottom tube) vortex 4mLs ice cold 70% Ethanol and slowly add the 200uL cells in PBS for rapid dispersion. Incubate on ice a minimum of 45 minutes. Overnight fixation (-20^{0}C) may be necessary for resolving sub-G_1 peak.

4. Centrifuge (pellet) cells (~1,200 rpm, 10', 4^{0}C).

5. Carefully aspirate supernatant and re-suspend cells in **PI Master Mix** (perform a final cell count) at a final cell density of 0.5X10^6 cells/mL. Incubate at 37^{0}C for 30 minutes prior to analysis by flow cytometry. Samples may be stored at 4^{0}C, in the dark for up to 3 days.

Reference

Ormerod, M.G., Ed.: Flow Cytometry A Practical Approach Second Edition
1992, Oxford University Press, Inc., New York, NY