Cell Cycle Analysis with Propidium Iodide

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Reagents:

80% EtOH (store at -20°C)

PI Solution:
0.1% Triton X-100 (stock 1%; **2 mls** for 20 mls final)
0.1 mM EDTA disodium (stock 10 mM; **200 ul** for 20 mls final)
50 ug/ml Rnase A (Sigma R-4875)(stock 10 mg/ml; **100 ul** for 20 mls final)
50 ug/ml PI (Sigma P-4170)(stock 1 mg/ml in PBS; **1 ml** for 20 mls final)
in PBS (**16.7 mls** for 20 mls final)

Protocol:

Keep cells on ice prior to use.
Aliquot $2x10^6$ cells into a FACS tube.
Wash 2X with cold PBS.
Decant the last wash and resuspend pellet prior to the addition of EtOH.
Resuspend cells in 1 ml 80% ice-cold EtOH while vortexing.
Incubate on ice a minimum of 30 minutes. (*Cells can be stored at 4°C in EtOH for several months*).
Wash cells 2X in cold PBS.
Resuspend cells on 0.5ml PI solution and incubate on ice until analysis.
Filter samples to remove aggregates immediately prior to analysis.