

Propidium Iodide Staining Procedure For Cell Cycle Analysis by flow cytometry

Fixation:

2×10^6 cells

1. Resuspend cell pellet
2. Add 1 ml 0.9% NaCl
3. Vortex gently and repeatedly while adding 2.5 ml 90% COLD EtOH dropwise
4. Fix at least 30 minutes at RT
5. Store at 4° C until ready to stain

Staining:

Stock Propidium Iodide - 500 $\mu\text{g}/\text{ml}$ made up in H_2O

Working Solution - 1:10 dilution in PBS

1. Spin cells out of fix
2. Resuspend pellet
3. Add 1ml of diluted Propidium Iodide
4. 30 minutes before flow cytometry analysis, add 100 μl of 1mg/ml Rnase.
5. Incubate at 37° C.
6. Samples are ready for flow cytometric analysis.

Propidium Iodide and RNase A can be purchased from Sigma Chemicals.
RNase must be DNase-free.