

Propidium Iodide Staining for FACS Analysis

1. Add 400 μ l (300 μ l) culture to 1.2 ml (0.9 ml) 100% ethanol in Eppendorf (volume not critical, therefore depends how much culture can be spared).
2. 1 h-2+ weeks at 4° C or -20° C.
3. Spin down cells.
4. Prepare n x 1 ml SSC (n = number of samples).
5. From that, prepare n x 0.25 ml SSC + 0.25 μ l RNaseA.
6. Resuspend cells in 250 μ l SSC RNase by vortexing.
7. Incubate at 50° C overnight (or at least 1 h if in a rush — but overnight gives better results).
8. Prepare n x 50 μ l SSC + 1.5 μ l proteinase K.
9. Add 50 μ l SSC protK to samples.
10. Incubate for exactly 1 h at 50° C.
11. Prepare n x 0.5 ml SSC + propidium iodide (final concentration is 3 μ g/ml).
12. Add 0.5 ml SSC propidium iodide to samples.
13. Incubate at room temperature for 1+ h then store at 4° C until use. (Overnight or over weekend gives best results but can store for at least a month and still get results.)