Propidium Iodide Cell Cycle Staining Protocol V.2

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Troubleshooting

1 Harvest cells in the appropriate manner and wash in PBS.

2 Fix in cold 70% ethanol (do not make this with PBS as it can cause protein precipitation during fixation). Add dropwise to the cell pellet while vortexing. This should ensure fixation of all cells and minimize clumping.

3 Fix for at least 30 minutes at 4°C. Specimens can be left at this stage for several weeks at -20°C. 00:30:00

4 Spin at 2000 rpm and be careful to avoid cell loss when discarding the supernatant, especially after spinning out the ethanol. Resuspend in 1xPBS, spin at 2000 rpm for two rounds of washes.

5 To ensure that only DNA is stained, treat cells with Ribonuclease. Add 50µl of 100µg/ml RNase.

6 Add 425µl of Cell Staining Buffer (Cat#**420201**) and 25 µl of Propidium Iodide Solution (Cat#**421301**).

Tip: Do not wash off PI after staining,