Propidium Iodide Cell Cycle Staining Protocol

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Abstract

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Protocol

Step 1.

Harvest cells in the appropriate manner and wash in PBS.

Step 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.

Step 3.

Fix for at least 30 minutes at 4°C. Specimens can be left at this stage for several weeks

DURATION
00:30:00

Step 4.

Wash x2 in PBS. Spin at 2000 rpm and be careful to avoid cell loss when discarding the supernatant, especially after spinning out the ethanol

Step 5.

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

Step 6.

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