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Propidium Iodide Cell Cycle Staining Protocol V.3

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1 Works for me dx.doi.org/10.17504/protocols.io.bacyiaxw

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EXTERNAL LINK

https://www.biolegend.com/protocols/propidium-iodide-cell-cycle-staining-protocol/4303/

GUIDELINES

General Tips and FAQ:

What is the expected result?

- The expected result for log-phase growing cells stained with PI should yield 2 distinct peaks on a histogram, with the lower peak corresponding to the G1 phase, and the second peak G2/M. DNA content in between is considered S-phase.

How do I set up my gates to distinguish the different cell cycle phases?

- As this distinguishes phases solely based on DNA content, gating for the 3 phases can be somewhat subjective. Certain flow cytometry data analysis softwares are equipped with automated gating options for PI-stained cells. Additionally, one may consider using controls to arrest cells in certain phases of the cell cycle, such as using a Thymidine block (G1/S), serum starvation (G0/G1) or Nocodazole (G2/M) to establish gating.

What can I do to better distinguish the distinct cell cycle phases?

- You can consider incorporating and staining for BrdU to your cells to better distinguish S-phase, and Ki-67 for M-phase. Check out the protocol for BrdU here and Ki-67 here.

MATERIALS

NAME	CATALOG #	VENDOR
Cell Staining Buffer	420201	BioLegend
Propidium Iodide Solution	421301	BioLegend

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.

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,