

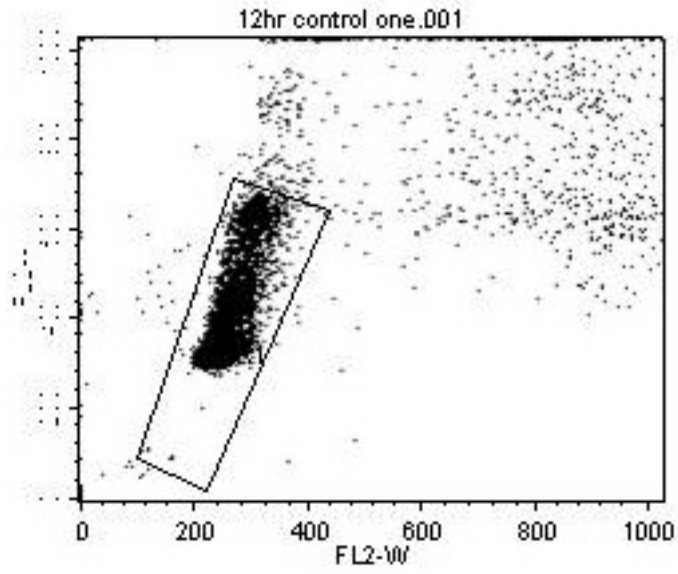
Cell Cycle Analysis by Propidium Iodide Staining

Prepared by: Prabodh Kandala

1. Plate 0.3×10^6 cells per well in a six well plate.
2. Final volume per well during the plating must be 2ml.
3. Incubate plate overnight in the incubator.
4. Following day, treat cells with respective treatments.
5. After specific time points, label the tubes accordingly.
6. Collect the media first, then trypsinize the cells,
7. Once the cells detach, collect all of them and centrifuge at 1200 rpm for 6 min.
8. Remove the supernatant, and wash the cells twice with PBS by centrifuging at 1200 rpm for 6 min.
9. Remove the traces of PBS. Then add 700 μ l of 100% ethanol and 300 μ l of PBS. Disperse the pellet and store the samples at 4⁰C.
(Note: These samples can be stored until 6 months)

10. On the day of experiment, centrifuge the sample at 1200 rpm for 6 min.
11. Remove the ethanol and wash cells twice with PBS for 1200rpm for 6 min.
12. Then add 500 μ l of PBS in each tube and disperse the cells.
13. Then add RNase at a final concentration of 0.5mg/ml.
14. Incubate at 37⁰C for 1 hour.
15. Add Propidium Iodide (PI) solution at a final concentration of 10 μ g/ml.
16. Shake it in the dark for 15min.
17. Take the readings on Flowcytometer.
18. In FACSCalibur, Draw a dot plot FL-2 W on X-Axis vs FL-2 A on Y-Axis.
19. Gate the population as shown below.

Sanjay Srivastava Lab Protocols



20. Now plot FL2-H histogram with the population gated.