Propidium Iodide Staining for DNA Content

Reagents: 95% Ethanol at -20°C
Propidium Iodide stock 1mg/mL in PBS (Sigma P4170)
RNase A Stock 1mg/mL in PBS (Sigma R5000)

Staining Solutions: 900µl 1x PBS + 2mM $MgCl_2$
50µl PI Stock Solution
50µl RNase Stock Solution
1ml total staining volume for ~$2x10^6$ cells

1. Wash cells in PBS to remove all traces of serum. Spin and aspirate supernatant.

2. Adjust cell concentration to $2x10^6$ cells/100µl in PBS.

3. Add 900µl of 95% ethanol, dropwise, to the cells while vortexing gently.

4. Store cells at 4°C to fix. Fix cells overnight, or ideally for 24 hours.

If the core will be processing your cells, you can stop the procedure and bring them at this stage. If you will be running them yourself, follow the rest of the protocol.

5. Spin cells in ethanol to pellet. Aspirate ethanol and wash once with PBS.

6. Spin cells again and aspirate most of the PBS, leaving a small amount to resuspend cells in.

7. Add 1ml of staining solution to each sample ($2x10^6$ cells) and incubate in the dark at 37°C for 20 minutes.

8. After incubation store cells on ice and analyze within a few hours.