

Cell Cycle Analysis

Cell isolation and Ethanol Fixation

1. Transfer 6-well plate from incubator to biosafety cabinet.
2. Remove supernatants and transfer to a labeled 15 mL conical.
3. Rinse wells with 0.5 mL PBS, then transfer rinse to the conical.
4. Add 0.5 mL trypsin to each well, incubate for 5 min at room temperature.
5. Quench trypsin with 1.5 mL DMEM. Transfer resulting cell suspension to the conical. [I typically collect 3 wells of a 6 well plate per sample, but this should be optimized for your samples.]
6. Spin down cell suspension at 1000g for 5 min, remove supernatant.
7. Resuspend pellet in 0.5 mL PBS.
8. Transfer resuspended pellet to 4.5 mL of chilled 70% EtOH while vortexing.
9. Incubate for 30 min on ice, then store at -20°C for at least 1 day.

Day of Flow Analysis

10. Prepare Propidium Iodide (PI) + RNAase Solution. Prepare 1 mL of solution for each sample.
For 10 samples:
 - 10 mL 0.1% TritonX in PBS
 - 200 uL RNAase (Thermo Scientific RNase #EN0531)
 - 200 uL PI (LifeTech #P3566)
11. Spin cell/ethanol mix at 1000g for 3 min.
12. Resuspend in 5 mL of PBS and let sit for ~30 sec.
13. Spin down at 1000g for 3 min.
14. Resuspend pellet in the above 1 mL PI-RNAase solution, and transfer to a FACS tube.
15. Store at room temp until analysis on flow cytometer (at least 2 hr).