CYTOMETRY RESEARCH, LLC

Cell Cycle Analysis

Materials: (Make sure buffers are ice cold as –4°C)

1. 12 X 75 mm polystyrene test tubes.
2. Propidium iodide stain (PI) (50 mg/ml) Sigma Catalog # P-4170, 10 mg, store at 2°-8°C.
3. Cells (1 X 10^6) in appropriate phases of the cell cycle suspended in PBS.
4. Ice cold ethanol (70%).
5. Stock solution of Rnase A (20 mg/ml). Store Frozen (-20°C). Sigma Catalog # R-6513, 250 mg, store at less than 0°C.

PROCEDURE:

Isolate cells:

1. For adherent cells, remove with PBS/EDTA and/or trypsin solution. For cells in suspension, harvest by centrifugation. Centrifuge cells at 1200 rpm at 4°C for 5 minutes, decant supernatant and gently re-suspend the cells in PBS.
2. Count the cells by hemocytometer.
3. Wash cells one time by putting 1X10^6 cells per tube, adding 1 ml of PBS and centrifuging at 1200 rpm at 4°C. Re-suspend pelleted cells in 0.3 ml of PBS buffer.

Fixing the cells:

1. To fix the cells, gently add 0.7 ml cold ethanol (70%) dropwise to tube containing 0.3 ml of cell suspension in PBS while vortexing gently.
2. Leave on ice for 1 hour (or up to a few days at 4°C).
3. Centrifuge cells as above, wash 1 time with cold PBS and re-centrifuge.
4. Re-suspend cell pellet in 0.25 ml of PBS, add 5 μl of 10 mg/ml Rnase A (the final concentration being 0.2-0.5 mg/ml).
5. Incubate at 37°C for 1 hour.
6. Add 10 μl of 1 mg/ml PI solution (the final concentration being 10μg/ml). Keep in the dark and at 4°C until analysis.
7. Analyze on FACS by reading on cytometer at 488 nm.

Propidium iodide stain (PI): Make a 1 mg/ml solution of PI in deionized water.