Fixation and Staining Cellular DNA (cell cycle)

Fixation

To quantitate cellular DNA content, cells must first be permeabilized by fixation, detergent lysis, or hypotonic shock; fixation with formalin is not recommended. Fixation with 70% ethanol for 30 min at +4°C is recommended (*see below). After permeabilization, cells should be washed in phosphate buffered saline (PBS) and resuspended at 10^6 cells/ml in PBS.

Always observe sterile technique (e.g., wear gloves and use sterile pipette tips) when handling RNase, DNase-free to avoid DNase contamination.

*For DNA content analysis preparation methods for cells from solid tissues, cells from solid tumors, and cells grown as monolayers or in suspension, see reference (Taylor, I.W (1980) J. Histochem. Cytochem. **28:** 1021).

Staining

Gloves & low light

1. Gently mix fixed sample
2. Spin to pellet cells (500 x g for 5-10 minutes) and remove supernatant
3. Wash cells by:
    a. Resuspend in 1 ml PBS
    b. Gently mix
    c. Spin to pellet and remove supernatant

    ***Determine "volumes" based on quantity of cells (size of pellet)
    - Large pellet = full volumes from protocol
    - Small pellet = ½ volumes from protocol
4. Resuspend in 1 ml or 0.5 ml PBS
5. Add 5.0 ul or 2.5 ul Rnase
6. Add 100 ul or 50 ul PI (Propidium Iodide) **light sensitive/carcinogenic**
7. Incubate at 37°C for 30 (covered with foil)
8. Mix
9. Put on ice for 15 min (covered with foil)

Propidium iodide
Roche Applied Science
Catalog # 11348639001
$83.00

RNase, DNase-free
Roche Applied Science
Catalog # 11119915001
$85.00