

CELL PREPARATION FOR DNA/CELL CYCLE STAINING

To quantitate cellular DNA content, cells must first be permeabilized by fixation, detergent lysis, or hypotonic shock; fixation with formalin is not recommended.

Fix cells with ethanol

1. Prepare the fixative by filling 12 x 75 mm-centrifuge tubes with 4.5 ml of 70% ethanol. Keep on ice.
2. Collect cells and suspend 10^6 to 10^7 cells in 5 ml PBS in a centrifuge tube.
3. Centrifuge cells 6 min at $\sim 200 \times g$ (e.g., 1000 rpm in Beckman TJ rotor).
4. Using Pasteur pipette thoroughly resuspend cells in 0.5 ml PBS.
It is important to achieve a single-cell suspension. Fixation of cells that are in aggregates while suspended in PBS stabilizes the aggregates, which are then impossible to disperse. It is essential, therefore, to have a monodisperse cell suspension at the time of mixing cells with ethanol.
5. Transfer the cell suspension into the tubes containing 70% ethanol. Fixation with 70% ethanol for 30 minutes @ 2-8°C is recommended.
6. Cells suspended in 70% ethanol can be stored @ 0-20°C for several months if not years.