DNA Staining in (Ethanol) Fixed Cells

Clinical or other specimens that cannot be immediately processed for DNA analysis (i.e. requirement of independent time collection of cells for their analysis) must to be fixed. A relatively easy method is ethanol fixation. Cells may be stored in fixative for extended periods and may be transported while in the fixative.

Ethanol is a precipitating fixative and also permeabilize cell membrane, aiding dye to DNA in intact cells and allowing the analysis of DNA content of stained cells by flow cytometry.

Ethanol Fixation

-Wash 1×10^6 to 1×10^7 cells with 1X PBS. Resuspend cell pellet in 0.5 ml 1X PBS. Add 3-4 ml of cold 70% Ethanol. Keep cells in ethanol for minimum of 2 hrs (4°C). Cells can be store in 70% ethanol at 0°C to -40°C for months (years).

-Centrifuge. Wash 1X with 1X PBS. Discard supernatant.

Propidium Iodide (PI) Staining:

-Resuspend cell pellet in 1.0 ml PI/Trixon X-100 solution with RNase A. Incubate 30 min/RT.

-Do not wash cells. Measure cell fluorescence in a flow cytometer (488 nm laser; 610/20 Bandpass filter).

✓ PI/Triton X-100 soln. w/RNase: to 10 mL of 0.1% (v/v) Triton X-100 in PBS add 2 mg DNase-free RNase A and 200 µL of 1 mg/ml PI. Prepare freshly.

✓ A stock solution of PI, made by dissolving 1 mg PI in 1 mL water, can be stored several months at 0°C to 4°C.

4',6-diamidino-2-phenylindole (DAPI) Staining:

-Resuspend cell pellet in 1.0 ml DAPI/Triton X-100 solution. Keep 30 min/RT/dark.

-Do not wash cells. Measure cell fluorescence in a flow cytometer (355 nm laser; 440/40 Bandpass filter; also works 405 nm laser; 450/50 BP filter).

✓ DAPI/Triton X-100 soln.: to 10 mL of 0.1% (v/v) Triton X-100 in PBS add 10 mg/ml DAPI. Prepare freshly.

✓ A stock solution of DAPI, made by dissolving 1 mg DAPI in 1 mL water, can be stored several months in dark (foil wrapped) at -20°C.

7-Aminoactinomycin D (7-AAD) Staining:

-Resuspend cell pellet in 0.5 ml of 25 µg/ml 7-AAD in 1X PBS. Incubate 15-30 min/RT/dark.

-Do not wash cells. Measure fluorescence in a flow cytometer (488 nm laser; 670/14 BP). Minimal spectral overlap between 7-AAD vs FITC and PE.