



شرکت سلول بافت زیست (سبز)

## DNA Content Analysis Protocol

1. Prepare the fixative by filling a centrifuge tube with 4.5 ml of 70% ethanol. Keep the tube on ice.
2. Collect cells and suspend  $10^6$  to  $10^7$  cells in 5 ml PBS in a centrifuge tube.
3. Centrifuge cells (200 g, 6 min)
4. Using a Pasteur pipette thoroughly resuspend cells in 0.5 ml PBS.\*
5. Transfer the cell suspension into the tubes containing 70% ethanol. Keep cells in fixative  $\geq 2$  hr.\*\*
6. Centrifuge the ethanol-suspended cells 5 min at 200 g. Decant ethanol thoroughly.
7. Suspend the cell pellet in 5 ml PBS, wait 60 sec, and centrifuge (200 g, 5 min)
8. Suspend cell pellet in 1 ml PI/Triton X-100 staining solution with RNase A. Keep either 15 min at 37°C or 30 min at room temperature.
9. Perform flow cytometry

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\* 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.

\*\*Cells suspended in 70% ethanol can be stored at 0° to -40°C for several months if not years.

### Materials:

-70% ethanol

-PBS

-Propidium iodide (PI)/Triton X-100 staining solution with RNase A:

To 10 ml of 0.1% (v/v) Triton X-100 in PBS add 2 mg DNase-free RNase A and 200  $\mu$ l of 1 mg/ml PI. Prepare freshly.

If the RNase is not DNase-free, boil a solution of 2 mg RNase A in 1 ml water for 5 min.

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

**Ref:** Current Protocols in Cytometry, Unit 7.5