

FIXATION PROTOCOL FOR APO-BRDU APOPTOSIS ASSAY

The following cell fixation procedure is a suggested method. Variables such as cell origin and growth conditions can affect the results. The fixation conditions provided below should be considered guidelines.

1. Suspend the cells in 1% (w/v) paraformaldehyde in PBS (pH7.4) at a concentration of $1-2 \times 10^6$ cells/ml.
2. Place the cell suspension on ice for 30-60 minutes.
3. Centrifuge cells for 5 min at 300 x g and discard the supernatant.
4. Wash the cells in 5 ml of PBS, then pellet the cells by centrifugation. Repeat the wash and centrifugation.
5. Resuspend the cell pellet in the residual PBS in the tube by gently vortexing the tube.
6. Adjust the cell concentration to $1-2 \times 10^6$ cells/ml in 70% (v/v) ice cold ethanol. Let cells stand for a minimum of 30 min on ice or in the freezer.
In some biological systems, storage of the cells at -20°C in 70% (v/v) ethanol for at least 12-18 hr prior to staining for apoptosis detection yields the best results.
7. Store cells in 70% (v/v) ethanol at -20°C until use.

***Cells can be stored at -20°C for several days to several months before use.**