

Cell Fixation Procedure for the APO-BrdU & APO-Direct Assays

NOTE: Cell fixation using paraformaldehyde is a **required** step in the **APO-BrdU & APO-Direct** assays. 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 as guidelines. Additional experimentation may be required to obtain results comparable to the control cells provided with this kit. The **positive** and **negative control cells** provided in the **APO-BrdU & APO-BrdU KITS** are already fixed.

1. Suspend $1-2 \times 10^6$ cells in 0.5 ml of 10 mM sodium phosphate pH 7.2, 150 mM sodium chloride (PBS).
 2. Add the cell suspension into 5 ml of 1% (w/v) paraformaldehyde in PBS and place on ice for 15 minutes.
 3. Centrifuge cells for 5 minutes at $300 \times g$ 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 cells in 0.5 ml of PBS.
 6. Add cells to 5 ml of ice-cold 70% (v/v) ethanol.
 7. Store cells in 70% (v/v) ethanol at -20°C until use.
- Cells can be stored at -20°C for several days before use.