# General fix, perm, and block protocol

This protocol provides general instructions for fixation, permeabilization, and block to prepare your cells for immunolabeling. Fixing and permeabilizing cells generally locks them in place and makes it possible for larger molecules such as antibodies to access the interior of the cell.

The volumes given in this protocol are good for a single well of adherent cells grown in a 6-well plate or a 35 mm dish.

## What you need

- Live cells
- PBS
- 4% Paraformaldehyde
- 0.5% Triton® X-100
- 3% w/v bovine serum albumin in PBS

| Step | Icon | Instruction |
|---|---|---|
| 1 | | Remove the medium from your cells (tip the vessel and pipet from the side). |
| 2 | | Add 1 mL of 4% (w/v) formaldehyde solution in phosphate-buffered saline (PBS). |
| 3 | 15 | Incubate for 15 minutes at room temperature for most targets. |
| 4 | | Remove the fixative solution and wash by pipetting PBS (a volume that will just cover the cells) against the side of the vessel, gently swishing the solution from side to side, and then tilting the vessel and removing the PBS; repeat 3 times. The fixed sample can be stored for several days at 5°C if needed. |
| 5 | | Add 1 mL of 0.5% Triton® X-100 (v/v) in PBS. |
| 6 | 15 | Incubate for 15 minutes at room temperature. |
| 7 | | Remove the permeabilization solution and wash 3 times with PBS as in step 4. |
| 8 | | Add 2 mL of 3% bovine serum albumin in PBS (or a different blocking solution if required). |
| 9 | 60 | Incubate for at least 60 minutes (up to overnight) at room temperature. |
| 10 | | Go to cell staining or antibody labeling. |

For more information, go to **lifetechnologies.com/imagingbasics**

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