

Ki-67 Staining Protocol by flow cytometry

1. Prepare target cells of interest and centrifuge 2X with Washing Solution, at 350xg for 5 minutes.
2. Carefully aspirate the supernatant so as not to touch the cell pellet.
3. While vortexing, add 3 ml drop by drop of cold 70% ethanol into the cells pellet. Then incubate at -20°C for 1 hour. These fixed cells can be used up to 60 days after fixing (Store at -20°C).
4. Add 30-40 ml of Staining Solution to the fixed cells.
5. Centrifuge the cells for 10 minutes at 350xg and aspirate supernatant. Wash twice and aspirate supernatant.
6. Resuspend the cells at the concentration of 0.5-10 x 10⁶ cells/ml.
7. Transfer 100 µl of cell suspension into 12x75mm cytometer tubes.
8. Add the suggested volume indicated on the antibody vial into the tubes above. Mix gently.
9. Incubate the tubes at room temperature (RT) for 20-30 minutes in the dark.
10. Wash with 2 ml of Washing Solution at 350xg for 5 minutes.
11. Carefully aspirate the supernatant so as not to touch the cell pellet.
12. Add 0.5 ml of Wash Solution into each tube. Add 5 µl of the appropriate exclusion non viable cells die into each tube and incubate for 5 minutes.

Acquire on a flow cytometer or store in the dark at 2 -8°C until the analysis is carried out.

Note: Ki-67 is expressed by proliferating cells. Using resting cells (eg, unstimulated PBMC) may give negative results.

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com)

Reagent list:

- Wash solution: 20 mM NaH₂PO₄, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN₃) + 0,5 % bovine serum albumin.
- Staining Solution: PBS 20 Mm, 150 NaCl with 1% FBS, 0.09% NaN₃ pH7.2
- Red blood cells lysing solution (if necessary)
- Flow cytometry solution: 20 Mm NaH₂PO₄, 150 NaCl, pH 7.2 + 1% Paraformaldehyde.
- Exclusion non viable cells PI Staining Solution (Ref. PI) for FITC conjugated antibody.
- Exclusion non viable cells 7-AAD Staining Solution (Ref. 7AAD) for PE conjugated antibody.