Immunofluorescence Protocol

Immunostaining of cultured cells using FluoTag® reagents.
For research use only - not intended for diagnostic use.

Materials (not included):

- Phosphate Buffered Saline (PBS) pH 7.4
- Paraformaldehyde (PFA): 4% PFA in PBS pH 7.4, freshly prepared
- Quenching Solution (QS):
  0.1 M Glycine in PBS pH 7.4
  or 0.1 M NH_4Cl in PBS pH 7.4
- Blocking and permeabilization buffer (BPB):
  10% Normal Goat Serum (NGS) + 0.1% Triton X-100 in PBS
  or 2% Bovine Serum Albumin (BSA) + 0.1% Triton X-100 in PBS
- FluoTag® Dilution Buffer (FDB):
  3% NGS + 0.1% Triton X-100 in PBS
  or 1% BSA + 0.05% Triton X-100 in PBS
- High-salt PBS: PBS supplemented with 0.5 M NaCl

∆***Notes:***

- *Below are two versions of the same IF protocol. The "Bench Protocol" is a concise version, while the "Detailed Protocol" provides an extended explanation.*
- *FluoTag® products are also compatible with methanol fixation. Fixation protocols using glutaraldehyde are not recommended.*
- *We recommend using blocking and FluoTag® Dilution Buffers prepared with Normal Goat Serum (NGS).*
- *To obtain optimal results for different target proteins and expression levels, the dilution factor might need to be adjusted. The recommended dilution specified in the data sheet is thus only a starting point for further optimizations.*

Bench Protocol: IF of cultured cells using FluoTag® reagents

1. Fix cells with 4% PFA for 30 min at room temperature (RT). FluoTag® reagents are also compatible with methanol fixation. In this case, step 2 can be omitted.
2. Quench with PBS supplemented with 0.1 M glycine or 0.1 M NH_4Cl for 10 min at RT.
3. Wash once with PBS.
4. Permeabilize and block for 15 min with 10% Natural Goat Serum (NGS) and 0.1% Triton X-100 in PBS.
5. Dilute FluoTag® with 3% NGS and 0.1% Triton X-100 in PBS. To obtain optimal results for different target proteins and expression levels, the dilution of FluoTag® products might need to be adjusted. The recommended dilution is thus only a starting point for optimization.
6. Incubate fixed cells with the diluted FluoTag® for 60 min at RT.
7. Wash 3 times for 5 min each with 1 mL of PBS.
8. Optional: Wash once with high-salt PBS (PBS + 500 mM NaCl) followed by PBS.
9. Shortly dip coverslip in water before mounting. We recommend using Mowiol as a mounting medium.

Detailed Protocol: IF of cultured cells using FluoTag® reagents

- The example below is based on a 12-well plate.
- Please adapt the protocol to your experimental conditions.

1. Wash cells gently using PBS (e.g. 1 mL of PBS per well).
2. Add 1 mL of 4% PFA per well and incubate for 30 min at room temperature (RT).
3. Remove PFA and dispose according your laboratory rules.
4. Briefly rinse with 1 mL QS per well.
5. Add 1 mL of fresh QS per well and shake gently on an orbital shaker (10 min, RT).
6. Remove QS.
7. Briefly rinse with 1 mL of PBS per well.
8. Add 1 mL of BPB per well and shake gently (15 min, RT).
9. During this time prepare the FluoTag® working solution. Make sure to prepare sufficient volume for all reactions (e.g. 5 mL for a full 12 well plate).
   a. Vortex FluoTag® stock solution shortly and centrifuge for 2 min at 10,000 x*g*.
   b. Dilute the FluoTag® reagent in FluoTag® Dilution Buffer.
10. Remove BPB solution from wells.
11. Add 400 µL per well of the FluoTag® working solution. Incubate for 60 min. with gentle shaking at RT and protected from light.
12. Remove the FluoTag® working solution from well.
13. Rinse once with 1 mL of PBS per well.
14. Wash with 1 mL of PBS per well and shake the plate gently for 5 min at RT and protected from light.
15. Repeat the previous step 2 times.
16. Optional step:
    a. Wash once for 5 min with high-salt PBS.
    b. Briefly rinse with PBS.
17. Before mounting, rinse once with water to remove the excess of salt.

For technical support contact information, visit: www.abcam.com/contactus

Version 1 | 2025-11-19