

Immunofluorescence Protocol

Immunostaining of cultured cells using fluorophores.
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 NH₄Cl 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
- Fluorophore Dilution Buffer:
 - 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

ΔNotes:

- Below are two versions of the same IF protocol. The "Bench Protocol" is a concise version, while the "Detailed Protocol" provides an extended explanation.
- These products are also compatible with methanol fixation. Fixation protocols using glutaraldehyde are not recommended.
- We recommend using Blocking and Fluorophore 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 fluorophore reagents

1. Fix cells with 4% PFA for 30 min at room temperature (RT). These 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 NH₄Cl 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 the fluorophore 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 fluorophore products might need to be adjusted. The recommended dilution is thus only a starting point for optimization.
6. Incubate fixed cells with the diluted fluorophore 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 fluorophore 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 fluorophore working solution. Make sure to prepare sufficient volume for all reactions (e.g. 5 mL for a full 12 well plate).
 - a. Vortex fluorophore stock solution shortly and centrifuge for 2 min at 10,000 xg.
 - b. Dilute the fluorophore reagent in Fluorophore Dilution Buffer.
 10. Remove BPB solution from wells.
 11. Add 400 µL per well of the fluorophore working solution. Incubate for 60 min. with gentle shaking at RT and protected from light.
 12. Remove the fluorophore 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.

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