Immunostaining of cultured cells using FluoTag® reagents

Materials (not included):

- Phosphate Buffered Saline (PBS) pH 7.4
- Paraformaldehyde (PFA):
  4% PFA in PBS pH 7.4, freshly prepared (1)
- Quenching Solution (QS):
  0.1 M Glycine in PBS pH 7.4
  or: 0.1 M NH_4Cl in PBS pH 7.4
- Blocking & Permeabilization buffer (BPB) (2):
  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) (2):
  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

Note: Below are two versions of the same IF protocol. The "Bench Protocol" is a concise version, while the "Detailed Protocol" provides an extended explanation.

Bench Protocol: IF of cultured cells using FluoTag® reagents

1. Fix cells with 4% PFA for 30 min at 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 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 FluoTag® with 3% NGS and 0.1% Triton X-100 in PBS.
   To obtain optimal results for different target proteins and expression levels, the diltion 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.

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Detailed Protocol: IF of cultured cells using FluoTag® reagents

Note:
- 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 at room temperature (30 min, 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 suffcient 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 (3).
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.

Remarks

(1) FluoTag® products are also compatible with methanol fixation. Fixation protocols using glutaraldehyde are not recommended.

(2) We recommend using blocking and FluoTag® Dilution Buffers prepared with Normal Goat Serum (NGS).

(3) 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.

For further information please contact us at info@nano-tag.com

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