Immunocytochemistry Protocol
- using protag single domain antibodies -

Solutions and reagents

10x PBS: 1.37 M NaCl
0.027 M KCl
0.1 M Na2HPO4
0.018 M KH2PO4

- Resolve in 800 ml ddH2O.
- Adjust pH to 7.4 using HCl.
- Fill it up to 1l.
- Autoclave it and store at room temperature.
- Dilute 1:10 before use.

High-salt PBS:
PBS supplemented with 0.5 M NaCl final concentration.

Paraformaldehyde (PFA)[(1)]:
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 & 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

protag Dilution Buffer (PDB):
3% NGS + 0.1% Triton X-100 in PBS
or: 1% BSA + 0.05% Triton X-100 in PBS

Remarks

(1) protag products are also compatible with methanol fixation. Fixation protocols using glutaraldehyde are not recommended.
(2) We recommend using blocking and protag 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.

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Procedure 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 protag working solution. Make sure to prepare sufficient volume for all reactions (e.g. 5 ml for a full 12 well plate).
10. Vortex protag stock solution shortly and centrifuge for 2 min at 10,000x g.
11. Dilute the protag reagent in protag dilution buffer [3].
12. Remove BPB solution from wells.
13. Add 400 µl per well of the protag working solution. Incubate for 60 min with gentle shaking at RT and protected from light.
14. Remove the protag working solution from well. oRinse once with 1 ml of PBS per well. oWash 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: Wash once with high-salt PBS (PBS + 500 mM NaCl) followed by PBS.
17. Shortly dip coverslip in water before mounting.
18. We recommend using Mowiol as a mounting medium.

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