Immunocytochemistry using protag sdAb

Solutions and reagents:

10x PBS:

1.37 M NaCl
0.027 M KCl
0.1 M Na_2HPO_4
0.018 M KH_2PO_4

- Resolve in 800 ml ddH_2O.
- Adjust pH to 7.4 using HCl.
- Fill it up to 1l.
- Autoclave it.
- Store at room temperature.
- Dilute 1:10 before use.

High-salt PBS:

PBS supplemented with 0.5 M NaCl final concentration

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 NH4Cl in PBS pH 7.4

Blocking & 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

protag Dilution Buffer (PDB):

3% NGS + 0.1% Triton X-100 in PBS
or: 1% BSA + 0.05% Triton X-100 in PBS

Procedure- 12 well plate

Please adapt the protocol to your experimental conditions.

- Wash cells gently using PBS (e.g. 1 ml of PBS per well).
- Add 1 ml of 4% PFA per well and incubate at room temperature (30 min, RT).
- Remove PFA and dispose according your laboratory rules.
- Briefly rinse with 1 ml QS per well.
- Add 1 ml of fresh QS per well and shake gently on an orbital shaker (10 min, RT).
- Remove QS.

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- Briefly rinse with 1 ml of PBS per well.
- Add 1 ml of BPB per well and shake gently (15 min, RT).
- 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).
- Vortex protag stock solution shortly and centrifuge for 2 min at 10,000x g.
- Dilute the protag reagent in protag dilution buffer.
- Remove BPB solution from wells.
- Add 400 µl per well of the protag working solution. Incubate for 60 min with gentle shaking at RT and protected from light.
- Remove the protag working solution from well.
- Rinse once with 1 ml of PBS per well.
- Wash with 1 ml of PBS per well and shake the plate gently for 5 min at RT and protected from light.
- Repeat the previous step 2 times
- Optional: Wash once with high-salt PBS (PBS + 500 mM NaCl) followed by PBS.
- Shortly dip coverslip in water before mounting.
- We recommend using Mowiol as a mounting medium.

Remarks

- protag products are also compatible with methanol fixation.
- Fixation protocols using glutaraldehyde are not recommended.
- We recommend using blocking and protag 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.

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