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Immunofluorescence staining

Materials

PBS with Ca^{2+} / Mg^{2+} (PBS $^{++}$):
add 25 µl 2.5M $CaCl_2$ and
50 µl 1M $MgCl_2$ to 50 ml PBS

Fixation buffer:
PBS
4% paraformaldehyde

BP (blocking/ permeabilization) buffer:
PBS $^{++}$
10% fetal calf serum
0.2% saponin

1st antibody
***tag*-tools** antibody
starting dilution 1:100;
different dilutions should
be tested: 1:50 – 1:500)

2nd antibody
secondary antibody
directed against 1st antibody,
fluorescence-labelled
(approx. dilution 1:200)

Mounting medium

Nail polish

Procedure

1. Cells are seeded on glass coverslips in 24-well plates
2. After gentle washing with PBS^{++} fix cells on coverslips with 500 µl fixation buffer/well for 10 min at room temperature
3. After fixation, wash the cover slips 3 x with 1 ml PBS^{++}
4. Add 300 µl/well BP buffer for 5 min at room temperature
5. Aspirate BP buffer and center the cover slip in the well
6. Add 200 µl of the first antibody (diluted 1:100 in BP buffer) in each well
7. Incubate 1 h at room temperature
8. Wash samples 3 x with 1 ml PBS^{++}
9. Incubate samples with 300 µl BP buffer for 5 min at room temperature
10. Add 200 µl of the fluorochrome-coupled secondary antibody (diluted 1:100 to 1:200 in blocking solution) in each well
11. Incubate for 45 min at room temperature in the dark
12. Wash samples 3 x with 1 ml PBS^{++}
13. Transfer cover slips onto glass slide with cell-side down on a 30 µl drop of mounting medium
14. After sucking off excess mounting medium, seal edge of cover slips with nail polish and keep samples dark
15. After nail polish has dried, analyse samples in the microscope