Immunocytochemistry of cultured cells

Solutions

Paraformaldehyde fixation solution (PFA)
4% paraformaldehyde
PBS (use TBS instead of PBS hereafter when an alkaline phosphatase is the marker enzyme)
Heat the solution (≤70°C) to dissolve paraformaldehyde. Note: Paraformaldehyde is hazardous!

Methanol or Acetone fixation
Ice-cold methanol
Ice-cold acetone

Blocking solution
1-3% Bovine Serum Albumin (and 10% goat serum)
0.1-0.3% Triton X100 or 0.5% TWEEN 20 (detergent is used when cells are fixed with PFA)
0.05% Sodium Azide (NaN_3, Sigma S-2002)
PBS

Antibody (Ab) solutions
Ab-solutions with desired concentrations of primary and secondary Ab are mixed in blocking solution. Add 250 µl Ab-solution per well in a 24 well cassette.

Procedure

If the cells are fixed with PFA use a detergent in the blocking solution from step 3 to 6. If cells are fixed with methanol or acetone the detergent can be omitted. Prepare coverslips with only the secondary Ab to check for unspecific immuno staining.

1. Wash with PBS or Ca^{2+} medium 2-3 times. Use Ca^{2+} medium if cells are pre-treated.
2. Fix cells with ice-cold PFA, methanol, or acetone fixation solution for 10 min.
3. Incubate with blocking solution for 30-60 min, shaking at room temperature (RT).
4. Incubate with primary Ab-solution 1 h to overnight, shaking at 4°C.
5. Wash with blocking solution 3 times (1→5→15 min), shaking at RT.
6. Incubate with secondary Ab-solution under aluminum foil for 1 h, shaking at RT.
7. Rinse with clean PBS 5 times (1→5→15→30→60 min) under aluminum foil, shaking at RT.
8. Mount the coverslips using Prolong Antifade Kit (Molecular Probes No. P-7481).
9. Leave the slides in dark RT overnight to dry the mounting medium.
10. Examine slides with microscope within a couple of days and then store slides in -20°C.

Unspecific background fluorescence can be reduced if using filtered blocking solution in step 5 and 6. Filter the solution with a syringe driven filter unit 0.22 µm (MILLEX-GS 33 mm).