Immunostaining Cultured Cells

All steps are carried out at room temperature unless otherwise indicated. For optimum staining, incubations should be carried out on a slow-moving rotary shaker unless the cell line being used is delicate (e.g. neuronal cells). Recipes for all solutions are listed at the end of the protocol.

Fixation and permeabilization

1. Aspirate medium, wash cells seeded on clean glass cover slips briefl y with 1X PBS.

2. Fix the cells with 4% paraformaldehyde made fresh in 1X PBS for 10 minutes. Rinse cover slips with 1X PBS 3 times for 3 minutes each.

3. Permeabilize with 0.2% Triton X-100 made in 1X PBS for 5 minutes. Rinse cover slips 3 times with 1X PBS for 3 minutes each.

Blocking

4. Prepare a blocking solution of 5% normal serum in 1X PBS. Select serum from the same species in which the secondary antibody was raised e.g. if the secondary antibody is goat anti-mouse, then goat serum should be selected for the blocking solution. Incubate the cells with the blocking solution for 1 hour.

(Alternatively, use 1% BSA in 1X PBS for blocking if the corresponding serum is not available.)

Antibody incubations

5. Aspirate the blocking solution and apply primary antibody diluted in antibody dilution buff er. Set aside one cover slip per experimental condition for a negative control and incubate in antibody dilution buffer minus the primary antibody. Leave these incubations for 1 hour, or, alternatively, incubate overnight at 4°C.

6. Wash cover slips with 1X PBS 3 times for 3 minutes each.

7. Apply an appropriate fl uorophore-conjugated secondary antibody diluted in antibody dilution buff er to the coverslips and incubate for 1 hour in a moist, dark environment. (NB: it is imperative that cover slips be kept in dark conditions as much as possible after the addition of fl uorescent reagents.)

8. Wash cover slips with 1X PBS 3 times for 3 minutes each.

Mounting and visualization

9. Mount cover slips on microscope slides with Hydromount (National Diagnostics) containing DAPI (if desired) for nuclear staining.

10. Examine slides under a fl uorescence microscope.

Solutions

1x PBS buffer (1000ml)

10mM Na_2HPO_4	1.42g
1.8mM NaH_2PO_4	0.22g
140mM NaCl	8.19g
2.68mM KCl	2ml
Adjust pH to 7.4	
Add ddH_2O to 1000 ml	

Antibody dilution buffer (20ml)

1% BSA 0.2g, add 1x PBS to 20ml

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