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IMMUNOSTAINING CULTURED CELLS

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All steps in this protocol are performed 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 (highlighted) in **bold** are included at the end of the protocol.

1. Fixation and permeabilization:

a. Aspirate medium, wash cells seeded on clean glass cover slips briefly with **1X PBS**.

b. 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.

c. 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.

2. Blocking:

a. 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.)

3. Antibody incubations:

a. Aspirate the blocking solution and apply primary antibody diluted in **antibody dilution buffer**. Set aside one cover slipper 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.

Please Note: If incubating overnight, take measures to ensure the cover slips do not dry out.

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

c. Apply an appropriate fluorophore-conjugated secondary antibody diluted in **antibody dilution buffer** to the coverslips and incubate for 1 hour in a moist, dark environment.

Please Note: It is imperative that cover slips be kept in dark conditions as much as possible after the addition of fluorescent reagents.

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

4. Mounting and visualization:

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

b. Examine slides under a fluorescence microscope.

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Solutions

1X PBS	In 1000 ml (final volume)
10 mM Na_2HPO_4	1.42 g
1.8 mM KH_2PO_4	0.24 g
137 mM NaCl	8 g
2.7 mM KCl	0.2 g
Adjust to pH 7.4	
Add ddH_2O to 1000 ml	

Antibody dilution buffer	In 20 ml (final volume)
1% BSA	0.2 g
Add 1X PBS to 20ml	

Related Proteintech Products

Product Name	Catalog Number	Size	Applications
FITC-conjugated AffiniPure goat anti-mouse Ig (G+L)	SA00003-1	100 µl	FC, IF
FITC-conjugated AffiniPure goat anti-rabbit Ig (G+L)	SA00003-2	100 µl	FC, IF
TRITC-conjugated AffiniPure goat anti-mouse Ig (G+L)	SA00007-1	100 µl	FC, IF
TRITC-conjugated AffiniPure goat anti-rabbit Ig (G+L)	SA00007-2	100 µl	FC, IF

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