Immunofluorescence Staining

Preparation of slides:

1. Grow cultured cells on sterile glass cover slips at 37°C overnight.
2. Wash sample with PBS twice.
3. Fix cells for 15 minutes with 2 mL of 4% paraformaldehyde solution (pH 7.4 with NaOH in PBS).

Step-by-step procedure:

1. Permeabilize cells by incubating for 15 minutes on ice with 2 mL of 0.1% Triton X-100 in PBS.
2. Wash cells 3 times with PBS.
3. Incubate cells for 1 hour with normal goat blocking serum (1:20 in PBS).
4. Introduce primary antibodies (in appropriate dilutions) to the sample.
5. Incubate for 4 hours at room temperature or at 4°C overnight.
6. Wash with PBS for 5 minutes. Repeat 5 times.
7. Incubate cover slips in fluorescein-conjugaed secondary antibodies in 5% normal blocking serum in PBS in a dark humidity chamber at 4°C for 1 hour. (Perform all subsequent washes under dim and ambient light source.)
8. Wash sample thoroughly with PBS. Each wash lasting 5 minutes. Repeat 6 times.
9. Counter stain sample with DAPI at room temperature for 30 minutes.
10. Wash sample with PBS. Each wash lasting for 2 minutes. Repeat 3 times.
11. Mount sample by inverting them onto mounting medium on glass slides.
12. Store slides in dark at 4°C.

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