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Product Information

Immunofluorescence Staining Procedure

Procedure

1. Grow cultured cells overnight on sterile glass cover slips at 37 °C.
2. Wash cells twice with PBS.
3. Fix cells for 15 minutes with 2 mL of PBS, pH 7.4, with 4% paraformaldehyde.
4. Permeabilize cells by incubating for 15 minutes on ice with 2 mL of PBS with 0.1% TRITON® X-100.
5. Wash cells 3 times with PBS.
6. Incubate cells for 1 hour with blocking solution (normal goat serum 1:20 [v/v] in PBS).
7. Introduce diluted primary antibody to the cells.
8. Incubate for 4 hours at room temperature or overnight at 2–8 °C.
9. Wash with PBS for 5 minutes. Repeat 5 times.
10. Incubate cover slips with secondary antibody-FITC conjugate diluted in blocking solution (5% [v/v] normal blocking serum in PBS) in a dark humidity chamber at 2–8 °C for 1 hour.
    Note: Perform all subsequent washes under dim and ambient light source.
11. Wash the sample thoroughly with PBS, with the wash lasting 5 minutes. Repeat the wash 6 times.
12. Counterstain sample with DAPI at room temperature for 30 minutes.
13. Wash the sample with PBS, with the wash lasting for 2 minutes. Repeat the wash 3 times.
14. Mount sample by inverting the cells onto mounting medium on glass slides.
15. Store the slides in the dark at 2–8 °C.

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