Protocol for IF/IHC

1. Rinse cells briefly in PBS (37℃, as quickly as possible).
2. Aspirate PBS, cover cells with 300~500 µl 4% formaldehyde in PBS.
3. Allow cells to fix for 15 minutes at room temperature.
4. Aspirate fixative, rinse three times in PBS for 1 minute each.
5. Block specimen in 5% FBS in PBS/Triton for 60 minutes.
6. While blocking, prepare primary antibody by diluting 100 X in PBS/Triton.

   (You will need 200~300 µl per coverslip.)
7. Aspirate blocking solution, apply diluted primary antibody.

   NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
8. Incubate overnight at 4°C.
9. Rinse 3X in PBS for 5 minutes each.
10. Incubate with fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.

    NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.
11. Rinse 3X in PBS for 5 minutes each.
12. Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store at 4°C in dark.

PBS/Triton: 1X PBS/0.3% Triton X-100

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