ADMEbio
for proteomic research

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Immunofluorescence (IF/IFC) Protocol

I. Materials and Reagents:

- Cell culture medium
- Chamber slide and cell culture plate
- 1X PBS (pH7.4)
- 1X PBST (0.05% Tween 20)
- Fixing solution: 4% Formalin/dd.H2O
- Penetration solution: 0.1% Triton X-100/1xPBS
- Blocking buffer: 0.3% BSA/1xPBS
- Primary antibody and Fluorochrome-conjugated second antibody
- Staining solutions: DAPI and Phalloidin
- Mounting medium
- Glass slides and tweezers
- Fluorescence microscopy

II. Experimental Procedure:

1. Cell pretreatment:
   - Wash cultured cells with PBS 2 times gently, 5 minutes per wash.
   - Fix by adding 0.2ml of 4% Formalin to each well, fix cells at RT for 20 minutes, then remove fixing solution and add approximately 0.5ml of 1X PBS to cover the cells.
   - Freeze cells at -20° (if proceeding, skip this step after fixing.)
   - Thaw treated cells at room temperature approximately 30 minutes.
   - Wash treated cells with 1X PBS 2 times, 5 minutes per wash.
2. Penetration: penetrate cells with 0.1% Triton X-100 at room temperature for 10 minutes.
3. Wash: wash penetrated cells with 1X PBS twice, each time for 5 minutes.
4. Block: block cells with 0.3ml of 0.3% BSA at room temperature for 30 minutes.
5. Primary antibody: remove the blocking solution, add primary antibody at recommended dilution into the well and incubate at 37°C for 60 minutes.
6. Wash: remove the primary antibody and wash cells with PBST 3 times, 5 minutes per wash.

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7. Secondary antibody: add diluted secondary antibody in blocking buffer at a desired concentration into cells, incubated at 37°C for 50 minutes. Avoid light by covering with foil paper.

8. Wash: remove the secondary antibody, wash cells with PBST 3 times, 5 minutes per wash.

9. Nuclei staining: add 0.2ml diluted DAPI solution at 10ug/ml into the well, and incubate at room temperature for 10 minutes (skip this step and go to step 13 if so desired).

10. Wash: remove the DAPI solution, wash cells with PBST 3 times, 5 minutes per wash.

11. Cytoplasmic actin staining: add 0.3ml diluted Phalloidin solution into the well, incubate at 37° C for 1 hour or 4°C overnight.

12. Wash: remove the Phalloidin solution and wash cells two times with PBST and one time with sterilized water, 5 minutes per wash.

13. Mount:
    - Use a clean glass slide to mark slide/name correctly.
    - Drop proper amount of mounting medium on the glass slide.
    - Take the chamber slide out from the well using tweezers, keep cells side down to cover on mounting medium of the glass slide.
    - Remove excess medium from the edge of chamber slide, try to avoid bubbles.

14. Visualization: observe cells using Fluorescence microscopy.