Immunofluorescence (IF) Protocol

Statement: The Immunofluorescence (IF) Protocol provided here is intended for reference only. Please design a more appropriate experimental protocol based on actual conditions.

1. Sample Preparation

Cell Culture: Seed cells on sterile coverslips in a culture dish and grow to 60-80% confluency.

2. Fixation

Fix cells with 4% paraformaldehyde in PBS for 10-15 minutes at room temperature .

Note: For some antigens, methanol fixation may be better (e.g., cytoskeletal proteins). Use instead of PFA.

Wash 3 times with PBS (5 minutes each).

3. Permeabilization (Optional)

(Skip if using methanol/acetone fixation)

Incubate with permeabilization buffer (e.g., 0.1-0.5% Triton X-100 in PBS) for 5-10 minutes at room temperature.

Wash 3 times with PBS (5 minutes each).

4. Blocking

Block nonspecific binding by incubating with blocking buffer (e.g., 5% normal serum from the species in which the secondary antibody was raised, or 1–5% BSA in PBS) for 1 hour at room temperature or overnight at 4°C.

No wash required before proceeding to primary antibody incubation.

5. Primary Antibody Incubation

Dilute primary antibody in blocking buffer (refer to antibody datasheet for optimal dilution).

Remove the blocking buffer from the samples and add the diluted primary antibody solution.

Cover the sample to prevent drying and incubate in a humidified chamber.

Incubate the samples at room temperature for 1-2 hours or at 4°C overnight for increased signal strength.

After incubation, wash 3-5 times with PBST (5 minutes each).

6. Secondary Antibody Incubation (Optional)

(Skip if using a fluorophore-conjugated primary antibody)

Dilute the fluorophore-conjugated secondary antibody in blocking buffer.

Protect from light and apply to the sample.

Incubate in a dark, humidified chamber for 1 hour at room temperature.

Wash 3-5 times with PBST (5–10 minutes each), protecting from light.

7. Nuclear Staining (Optional)

Incubate with DAPI or Hoechst in PBS for 5-10 minutes at room temperature.

Wash 3 times with PBS (5 minutes each).

8. Mounting (Optional)

Mount coverslips onto slides using antifade mounting medium.

Seal edges with nail polish to prevent drying.

9. Imaging

Store slides in the dark at 4°C until imaging.

Examine the samples using a fluorescence microscope equipped with appropriate filters for the fluorophores used (e.g., FITC, Cy3, Cy5, DAPI).

Capture images at different magnifications (e.g., 20×, 40×, 63×, 100×) as required.

10. Data Analysis

Analyze the acquired images using image analysis software (such as ImageJ) to quantify fluorescence intensity, measure the size and number of positive cells, or study the subcellular localization of the target protein.

Notes

- *Always include appropriate controls (e.g., no primary antibody control, isotype control).*
- *Optimize antibody concentrations empirically.*
- *Avoid prolonged exposure to light during all fluorescent steps to minimize photobleaching.*
- *For multicolor staining, ensure spectral compatibility between fluorophores and use secondary antibodies from different host species.*