www.rockland.com
tech@rockland.com
+1 484.791.3823

Protocol

Immunofluorescence (IF)

Immunofluorescence microscopy is used to localize specific constituents in tissue sections or immobilized cells using fluorescent tags as labels. The antibody/antigen complex is labeled with any of a variety of fluorochromes emitting light from the near UV to the near IR. Usually, FITC, a fluorescein derivative, is used for routine work and shows a yellow/green color. Red is often visualized using Texas Red, rhodamine, or Cy3. Blue occurs when AMCA or Cascade Blue is used. Fluorescent microscopes contain limit filters that are optimized for each fluorochrome. Confirm that your microscope is optimized for each dye you plan to use in your experiment. Black and white, color photography, or enhanced imaging are often used to record data.

Reagents Required

Product	Preparation	Item No.
Phosphate Buffered Saline (PBS)	Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 M NaCl). Dilute appropriate volume to 1X with deionized water.	MB-008
Formaldehyde Fixative	Dilute to 1% in PBS buffer. Use high-quality formaldehyde.	
Permeabilization Buffer	Prepare an appropriate volume of 0.2% (v/v) Triton X-100 in PBS buffer.	
Antibody Dilution Buffer	Prepare 100 mL of PBS Wash Buffer supplemented with 1 mL of normal serum of the same species as the host used for the secondary antibody.	
Fluorescein Secondary Antibody	Prepare dilution of fluorescein-conjugated secondary antibody (1:200) in Antibody Dilution Buffer. Use secondary antibody conjugate against the same species as the primary antibody.	
Bis-Benzimide Solution	Prepare reagent at 0.5 mg/mL in PBS buffer.	
Aqueous Mounting Media		

Procedure

1. Grow cells on sterile 12 mm glass coverslips placed in 24-well culture plates. Remove culture medium.
2. Gently wash cells 3 times with ice-cold PBS for 5 minutes per wash.
3. Fix cells by adding a volume of 1% formaldehyde in PBS equal to the original volume of culture medium. Incubate on ice for 5 minutes. Remove the fixative and wash as in Step 2.
4. Permeabilize cells for 20 minutes on ice with permeabilization buffer.
5. Prepare primary antibody appropriately diluted in antibody dilution buffer. React cells with primary antibody for 1 hour at room temperature.
6. Wash as in Step 2.

7. Prepare dilution of fluorescein-conjugated affinity-purified secondary antibody. React cells for 1 hour at room temperature with reagent. Alternatively, use a biotin-conjugated secondary antibody followed by a wash as in Step 2. Add fluorescein-conjugated streptavidin diluted 1:200 in PBS buffer. React for 30 minutes at room temperature.
8. Wash as in Step 2.
9. Counterstain cells with bis-benzimide solution for 15 minutes at room temperature.
10. Wash as in Step 2.
11. Add aqueous mounting agent. Affix coverslips to slides. Allow coverslips to dry in the dark before viewing.

References

- Ascoli, C. A., & Maul, G. G. (1991). Identification of a novel nuclear domain. Journal of cell biology
- Čeřovská, N. (1995). Antibody Techniques. Biologia plantarum