General Protocol

Immunofluorescence Kit

For Research Use Only

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Introduction

This kit is designed to stain a specific peptide antigen in fixed tissue by an indirect immunofluorescence technique. The indirect immunofluorescence technique uses the primary antibody directed against the specific antigen (peptide) in the tissue followed by a secondary antibody, which binds to the primary antibody and is conjugated with a fluorescent dye, such as fluorescein-isothiocyanate (FITC). Positive staining will show an emission of fluorescent light (FITC: green to yellow) in a dark field. We recommend the use of frozen tissue sections. The kit provides sufficient reagents for 50 slides.

Materials Provided

- Primary antibody: peptide-specific rabbit (or guinea pig) antiserum, 10 ml (1:200) (lyophilized powder).
- Secondary antibody: fluorescent dye-conjugated goat antibody directed against rabbit (or guinea pig) immunoglobulins, 100 µl.
- Negative control: normal rabbit (or guinea pig) serum, 5 µl (lyophilized).
- Phosphate buffered saline (PBS) concentrate (5x), 10 ml, pH 7.4.
- 3% Triton X-100/water, 5 ml.
- Mounting media: Glycerin-PBS (9:1), 2 ml.
- Normal goat serum (NGS, 1:10), 13 ml (lyophilized powder).

Materials Required but not Provided

- Rinsing buffer: 0.1 M phosphate buffered saline (PBS), pH 7.4, containing: 19 mM sodium phosphate monobasic, monohydrate; 81 mM sodium phosphate dibasic, anhydrous; 0.05 M sodium chloride. Store at 4 °C.
- Glass microscope slides coated with gelatin; cover slips.
- Incubation chamber or dishes.
- Cryostat.
- O.C.T. (Tissue-Tek, Miles Laboratories, Inc.) embedding medium.
- Dark field microscope (using transmitted or reflected light) with filters which are appropriate for FITC detection (excitation at 440-490 nm, emission at 520-560 nm).

Preparation Procedures

A) Rehydrate 0.1 M PBS with 45 ml of distilled water. Add 5 ml of 3% Triton X-100/water to the PBS solution. This will give 50 ml of 0.1 M PBS containing 0.3% Triton X-100.
B) Rehydrate primary antiserum with 10 ml of 0.1 M PBS containing 0.3% Triton X-100. This will give 10 ml of primary antiserum (1:200), which is sufficient for 50 slides using 200 µl of antiserum per slide.
C) Rehydrate NGS with 13 ml of 0.1 M PBS containing 0.3% Triton X-100. This will give 13 ml of NGS (1:10).
D) Rehydrate normal rabbit serum with 1 ml of 0.1M PBS. This will give 1 ml of NRS at a concentration of 1:200.
E) Dilute the fluorescent secondary antibody with 9.9 ml of 0.1 M PBS containing 0.3% Triton X-100. This will give 10 ml of the fluorescent secondary antibody (1:100).

Staining Procedure

Day 1

A) Bring the frozen tissue sections to room temperature and defrost for 5 minutes.
B) Wash slides 3 times for 10 minutes each with 0.1 M PBS.
C) Blot excess PBS from the slides with an absorbent wipe **(Do not touch the tissue or allow it to dry).**
D) Apply 200 µl of NGS (1:10) on the tissue sections. Incubate the slides at room temperature for 30 minutes in a closed incubation chamber.
E) Blot slides **(Do not touch the tissue or allow it to dry)**.
F) Apply approximately 200 µl of the primary antiserum (1:200) or negative control on tissue sections. Incubate the slides at 4 °C for 18-24 hours in the same chamber.

Day 2

A) Wash the slides 3 times for 10 minutes each with 0.1 M PBS.
B) Blot the excess PBS from the slides with an absorbent wipe **(Do not touch the tissue or allow it to dry)**.
C) Apply 200 µl of the fluorescent secondary antibody (either goat anti-rabbit or anti-guinea pig IgG) (1:100). Incubate the slides at room temperature for 60 minutes in the same chamber.
D) Wash the slides 3 times for 10 minutes each with 0.1 M PBS.
E) Drain and blot the excess PBS **(Do not touch the tissue or allow it to dry)**. Coverslip with glycerin-PBS (9:1).

F) Examine the stained tissue sections under a dark field microscope for the presence of a specific fluorescent staining pattern.

Summary of Immunofluorescence Technique

Defrost slides (5 min.)
↓
Wash slides (0.1 M PBS) (3 x 10 min.)
↓
NGS (1:10) (30 min.)
↓
Primary antiserum (18-24 hrs.)
↓
Wash slides (0.1 M PBS) (3 x 10 min.)
↓
Secondary antibody (goat anti-rabbit or -guinea pig IgG-FITC) (60 min.)
↓
Wash slides (0.1 M PBS) (3 x 10 min.)
↓
Coverslip (glycerin-PBS)
↓
Examine slides with a dark field microscope

This procedure is not intended to replace the preparation steps, precautions and procedures shown in this protocol. Please consult those sections for complete directions.

Technical Notes

A) Storage: 4 °C.
B) Expiration date: 3 months.
C) Tissue fixation: 4% paraformaldehyde in 0.1 M PBS, pH 7.4.
D) Dilution: The 1:200 dilution of the primary antibody is suitable for most antisera. However, if the background is too high or if the staining is either too strong or unclear, the antibody can be diluted further.
E) Crossreactivity: Use the same procedure as in the negative control. The concentrations of the tested peptides are 1-10 μM.

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