

← All Protocols

Fluorescent Antibody Detection Protocol

Overview

Materials

Protocol

Imaging Tips

Troubleshooting

Protocol Overview

Immunofluorescence (IF) is a technique that uses fluorescently-labeled antibodies to detect specific proteins or antigens in tissue sections. This protocol is designed for both paraffin-embedded and cryosectioned tissue samples.

Duration

Day 1: ~3 hours (+ overnight incubation)

Day 2: ~2 hours

Total: 2 days

© Key Steps

- 1. Deparaffinization/Antigen retrieval
- 2. Blocking (1 hour)
- 3. Primary antibody (overnight)
- 4. Secondary antibody (1 hour)
- 5. DAPI and mounting



Important Notes:

- Protect slides from light after adding fluorescent antibodies
- Work quickly during mounting to prevent drying
- Store stained slides at 4°C protected from light
- Image slides within 1-2 weeks for best results



Safety Information

- Always work with xylene in a fume hood (for paraffin sections)
- Wear appropriate PPE (lab coat, gloves, safety glasses)
- Handle antibodies with care some may contain sodium azide preservative
- Dispose of fluorescent waste appropriately
- Be cautious with hot liquids during antigen retrieval
- Follow proper storage conditions for antibodies (typically 4°C or -20°C)

↑ Back to Top

About This Protocol

Immunofluorescence (IF) staining protocol for detecting specific proteins using fluorescently-labeled antibodies in tissue sections.

Quick Links

← All Protocols

Protocol Overview

Troubleshooting

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