



# Immunohistochemistry (IHC)

DAB-Based Antibody Detection Protocol

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## Protocol Overview

Immunohistochemistry (IHC) is a technique that uses enzyme-labeled antibodies to detect specific proteins or antigens in tissue sections. This protocol uses the ABC (Avidin-Biotin Complex) method with DAB (3,3'-Diaminobenzidine) chromogen for brown colorimetric detection.



### Duration

**Day 1:** ~4 hours (+ overnight incubation)

**Day 2:** ~4 hours

**Total:** 2 days



### Key Steps

1. Deparaffinization & antigen retrieval
2. Peroxide quench & blocking
3. Primary antibody (overnight)
4. Secondary antibody & ABC
5. DAB development & counterstain



Important Notes:

- Prepare ABC solution at least 30 minutes before use
- Prepare DAB immediately before use - it's light-sensitive
- Monitor DAB development under microscope to prevent overstaining
- DAB is a potential carcinogen - handle with care and dispose properly



### Safety Information

- **DAB is a potential carcinogen** - always wear gloves, work in fume hood, dispose as hazardous waste
- Always work with xylene in a fume hood - fumes are toxic
- Wear appropriate PPE (lab coat, gloves, safety glasses)
- Handle antibodies with care - some may contain sodium azide preservative
- H<sub>2</sub>O<sub>2</sub> can cause burns - handle with care
- Be cautious with hot liquids during antigen retrieval
- Follow proper storage conditions for antibodies (typically 4°C or -20°C)

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## About This Protocol

Immunohistochemistry (IHC) staining protocol using ABC method with DAB chromogen for detecting specific proteins in tissue sections.

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