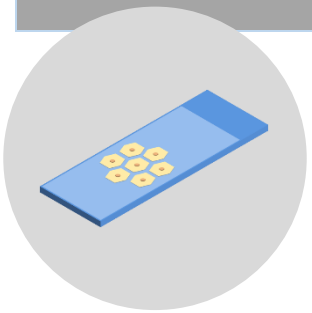
Davids Protocols



Immuno-Histo-Chemistry
IHC for frozen tissue

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Immunohistochemistry (IHC) is a technique employed to detect and visualize specific proteins or antigens in tissue samples. It provides insights into the presence and distribution of the antigens. There are two common approaches to perform IHC. One method involves working with paraffin-embedded tissue samples, where the tissue is fixed, dehydrated, and embedded in paraffin wax. The second approach, that is used in this protocol, involves using frozen tissue samples. The tissue is rapidly frozen using techniques such as snap freezing. Afterwards, tissue sections are cut, mounted on slides and processed for IHC staining.

- 2 - Material

Material		
Blocking Buffer	5% Sera from the same species as the secondary antibody (use normal chicken IgY for chicken anti-rabbit antibody) Alternatively: 5% BSA	
DAB Substrate	0.05% DAB 0.02% Hydrogen peroxide	
Mounting Solution	100 ml H_2O 10 ml Glycerol 10 ml PEG 400 (polyethylene glycol) 1 g PVA (polyvinyl alcohol)	
Washing Step	1 x TBS **or** 1 x PBS 3% Hydrogen peroxide	
Dehydration Steps	60% Ethanol 80% Ethanol 95% Ethanol 100% Ethanol 100% Xylene	
Primary Antibody		
Secondary Antibody	Conjugated anti-species antibodies	www.davids-science.de

- 3 - Method

Sample Preparation

- Use freshly dissected tissue block, ensuring it is less than 5 mm thick
- Place the tissue block onto a base mold
- Thoroughly soak the entire tissue block with Mounting Solution
- Transfer the mold with the tissue block into liquid nitrogen until it is completely frozen
- Store the frozen tissue block at -80°C for long-term storage
- Prior to sectioning, allow the temperature of the tissue block to equilibrate to -20°C
- Slice the tissue into desired segments, ensuring each segment is less than 10 µm thick
- Transfer the tissue segments onto microscope glass slides
- Allow the tissue segments to air-dry at room temperature
- Store the segments in a slide box at -80°C for long-term storage

Wash Step

- Immerse microscope glass slides with tissue segments in acetone at -20°C for 10 minutes
- Rinse the slides and allow the acetone to evaporate from the tissue segments for at least 20 minutes at room temperature
- Wash the tissue segments two times in 1x TBS for 5 minutes each
- Incubate the segments with 3% H_2O_2 in distilled water for 10 minutes to quench endogenous peroxidase activity
- Rinse the segments three times with 1x TBS for 3 minutes each
- Transfer the segments into a staining container
- Incubate the segments in Blocking Buffer for 1 hour
- Drain off the Blocking Buffer

Staining

- Incubate the tissue segment in a humidified chamber with the appropriately diluted primary antibody in 1x TBS for 1 hour
- Rinse the tissue three times in 1x TBS for 3 minutes each
- Incubate the tissue segment in a humidified chamber with the appropriately diluted biotinylated secondary antibody in 1x TBS for 30 minutes
- Rinse the tissue three times in 1x TBS for 3 minutes each
- You may want to incubate the tissue in a humidified chamber with the appropriately diluted streptavidin-HRP conjugate in 1x TBS for 30 minutes
- Rinse the tissue three times in 1x TBS for 3 minutes each

Substrate

- Incubate the tissue with freshly made DAB substrate solution until color development occurs, for 5-8 minutes
- Rinse the segments three times with distilled water for 3 minutes each
- You may want to immerse the segments in a hematoxylin bath for 3 minutes to stain the cell nuclei
- Rinse the segments with distilled water for 10 minutes

Microscopy

- Dehydrate the tissue segments using sequential 60%, 80%, and 95% ethanol for 5 minutes each
- Dehydrate the segments two times in 100% ethanol for 5 minutes each
- Soak the segments two times with fresh xylene for 5 minutes each
- Soak the segments in Mounting Solution and allow the tissue to air-dry
- Observe the staining in the tissue under microscopy

- 4 - Trouble Shooting

Reason	Solution
No Staining	Try using a stronger fixative to improve staining. Maintain a low storage temperature for the samples to preserve the staining quality.
No-Specific Staining	Increase the concentration of the Blocking Buffer to reduce nonspecific staining. Use a blocking buffer that contains sera from the same species as the secondary antibody to improve specificity.
Faint Staining	Use a higher concentration of the antibodies to enhance staining intensity. Increase the incubation time on the tissue to allow for better antibody binding and signal amplification.
High Background	Ensure thorough washing steps to reduce background staining. Decrease the concentration of antibodies to minimize nonspecific binding and background signal.