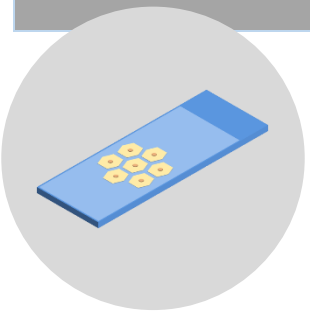

Immuno-Histo-Chemistry
IHC for paraffin-embedded tissue

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. The first method that is used in this protocol, involves working with paraffin-embedded tissue samples, where the tissue is fixed, dehydrated, and embedded in paraffin wax. The second approach 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	
Sample Preparation	10% Formalin Paraffin
De-/Hydration Steps	60% Ethanol 80% Ethanol 95% Ethanol 100% Ethanol 100% Xylene
HIER/Washing Step	3% Hydrogen-Peroxide 10 mM Sodium Citrate, pH 6.0 1 x TBS **or** 1 x PBS
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)

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Primary Antibody		
Antigen Retrieval, PIER	Proteinase K	https://davids-science.de/ProteinaseK
Secondary Antibody	Conjugated anti-species antibodies	https://davids-science.de/Secondaries

- 3 - Method

Sample Preparation

- Use freshly dissected tissue block, ensuring it is less than 5 mm thick
- Preserve the tissue with 10% Formalin at room temperature for 24 – 48 hours
- Rinse and incubate the tissue block with distilled water for 1 hour
- Dehydrate the tissue block using sequential 60%, 80%, 95% and 100% ethanol for 40 minutes each
- Dehydrate the segments two times in 100% xylene for 1 hour
- Immerse the tissue block three times in paraffin for 1 hour each
- Place the paraffin-embedded tissue block onto a base mold
- 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 2 – 8°C for long-term storage

Rehydration/Deparaffinization

- Soak the tissue slides two times in 100% xylene for 10 minutes each
- Place the segments two times in 100% ethanol for 5 minutes each
- Rehydrate the tissue using sequential 95, 80, 60% ethanol for 5 minutes each
- Rinse the deparaffinized tissue segments in distilled water three times for 3 minutes each

Antigen Retrieval

- Soak the tissue slides in sodium citrate buffer
- Boil the slides in a water bath for 10 minutes
- Let the tissue segments cool down at room temperature for 40 minutes
- Rinse the tissue three times with 1x TBS for 3 minutes each
- Incubate the tissue slides with 3% hydrogen peroxide for 10 minutes
- Rinse the tissue three times with 1x TBS for 3 minutes each
- Incubate the tissue slides in Blocking Buffer for 1 hour
- Drain off the Blocking Buffer, ensuring that the tissue segments do not dry out

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 slides 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

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- Incubate the tissue segments 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
Rehydration Step	Do not allow the tissue segments to dry out at any point after deparaffinization
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.
Antigen Retrieval	Ensure using the right buffer depending on the primary antibody used. For phospho-tyrosine specific antibodies please use an EDTA buffer instead of a sodium citrate buffer. Consider using PIER (Protease-Induced Epitope Retrieval) which utilizes enzymes (e.g. Proteinase K, Pepsin or Trypsin) as an alternative to HIER (Heat-

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Reason	Solution
	Induced Epitope Retrieval) to enhance epitope recovery and staining effectiveness. Ensure using the right enzyme and reaction conditions, indicated on the antibody product datasheet.

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