DAVIDS PROTOCOLS

Davids Biotechnologie

Immuno-Histo-Chemistry (IHC)

For frozen tissue

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General Information

Immunohistochemistry (IHC) is used to determine the presence and distribution of certain proteins or antigens in tissue samples. IHC is frequently utilized in histology, pathology, and other fields of biology and medical research. There are two ways to use IHC. The first involves paraffin-embedded tissue, whereas the second utilizes frozen tissue.

Materials

Blocking Buffer	DAB Substrate	Mounting Solution	Primary Antibody
Normal Sera 5% (must originate from same species used in secondary antibody) Alternatively: 5% BSA	0.05% DAB 0.02% Hydrogen peroxide	100 mL A. dest 10 mL glycerol 10 mL polyethylene glycol (PEG) 400 1 g polyvinyl alcohol (PVA)	www.davids-bio.com: For rabbit antibodies For chicken antibodies For mouse antibodies

Material needed for IHC		Method specific Chemicals
• Formalin 10% • Ethanol (60, 80, 95 and 100%) • Xylene • Hydrogen peroxide 3% • 1x TBS or 1x PBS	• A. dest • Biotinylated secondary antibody • Streptavidin-HRP conjugate • Hematoxylin	Frozen-embedded Method: • Acetone -20°C • Liquid nitrogen

Sample Preparation

- Use freshly dissected tissue block with a thickness of less than 5 mm
- Place block onto a base mold
- Soak whole tissue block with mounting solution
- Place mold with tissue block into liquid nitrogen until completely frozen
- Longtime storage of frozen tissue block at -80°C
- Equilibrate the temperature of tissue block to -20 °C before sectioning
- Slice the tissue into desired segments with a thickness of less than 10 µm
- Transfer tissue segments onto microscope glass slides
- Allow tissue segments to air-dry at room temperature.
- Longtime storage of segments in a slide box at -80°C

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Staining

- Immerse microscope glass slides with tissue segments in acetone at -20°C for 10 min
- Rinse and allow acetone to evaporate from tissue segments for at least 20 min at room temperature
- Wash the tissue segments two times in 1x TBS for 5 min each
- Incubate segments with 3% h2o2 in distilled water for 10 min (quench endogenous peroxidase activity)
- Rinse segments three times with 1x TBS 3 min each
- Transfer segments in a staining container

- Recommended step:
- Incubate segments in blocking buffer for 1 h
- Drain off blocking buffer

Incubation with Antibodies

- Incubate tissue segment in a humidified chamber with appropriately diluted primary antibody in 1x TBS for 1 h
- Rinse the tissue three times in 1 x TBS, 3 min each
- Incubate tissue segment in a humidified chamber with appropriately diluted biotinylated secondary antibody in 1x TBS for 30 min
- Rinse tissue three times in 1 x TBS, 3 min each

- Recommended step:
- Incubate the tissue in a humidified chamber with appropriately diluted streptavidin-HRP conjugate in 1xTBS for 30 min
- Rinse tissue three times in 1xTBS, 3 min each

Incubation with Substrate

- Incubate tissue with freshly made DAB substrate solution until color development for 5 – 8 min
- Rinse segments three times with distilled water, 3 min each

- Recommended step:
- Immerse segments in hematoxylin bath for 3 min to stain cell nuclei
- Rinse segments with distilled water for 10 min

Dehydration and Observation

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

DAVIDS TROUBLESHOOTING

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TROUBLESHOOTING

Reason	Solution
No Staining Due to a lack of antigenicity of the proteins in the tissue	Implement a stronger fixative Maintain a low storage temperature Due inadequate fixation of the tissue or degradation of proteins during processing or storage
No-Specific Staining Due to the binding of primary or secondary antibodies to non-specific protein in the tissue	Implement a higher concentration of Blocking Buffer Using a blocking buffer with sera of the same species as the secondary antibody
Faint Staining Due to a low concentration of the primary or secondary antibody	Using a higher concentration of the antibodies Increasing the incubation time on the tissue
No-Specific Staining Due to the binding of the primary or secondary antibodies to multiple proteins in the tissue	Implement a more specific antibody to the protein of interest Using a different species of secondary antibody that contains less cross-reactivity with the tissue
High Background Staining Due to the presence of excess primary or secondary antibodies in the tissue	Maintain a more thoroughly washing steps Using a lower concentration of antibodies
Optimize Antibody contidions	Implement a titration of both primary and secondary antibodies
Optimize Comparability	Implement a positive control
Hard to recover Epitopes	Using PIER (Protease-Induced Epitope Retrival) as an alternative to HIER (Heat-Induced Epitope Retrival) can increase effectiveness