

Immunohistochemistry (IHC)

Description:

IHC is used to understand the distribution and localization of proteins in different parts of a biological tissue. IHC detects specific antigens in preserved tissue sections using an appropriate antibody labeling strategy. Samples are collected, fixed to maintain cell morphology, tissue architecture and antigenicity of target epitopes, and then sectioned. A variety of antibody staining schemes can produce informative IHC images.

Protocol for Fixed and Paraffin Embedded Tissues with Sodium Citrate Antigen Retrieval:

Formalin or other aldehyde fixation form protein cross-links that mask the antigenic sites in tissue specimens, leading to weak or false negative staining for immunohistochemical detection of certain proteins. Sodium citrate treatments breaks the protein cross-links, unmasking the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, enhancing staining intensity of antibodies.

Deparaffinization: At Room Temperature

- 5 minutes each: wash slides in 3 changes of xylene

Rehydration: At Room Temperature

- 3 minutes each: wash slides in 3 changes of 100% alcohol
- 3 minutes each: wash slides in 2 changes of 95% alcohol
- 3 minutes each: wash slides in 1 change of 80% alcohol
- 5 minutes: Gently rinse slides using distilled water

Antigen Retrieval:

- At 99-100°C for 20 minutes: steam slides in 0.01 M sodium citrate buffer, pH 6.0
- At Room Temp for 20 minutes: remove slides from heat and let cool in buffer
- At Room Temp for 1 minute: Rinse in 1x TBS with Tween (TBST)

Immunostaining: Tissues should not dry at any time during the procedure!

- At Room Temp for 20 minutes: apply universal protein block
- Drain the protein block from the slides
- 45 minutes at Room Temp: Apply (diluted) primary antibody
- 1 minute at Room Temp: Rinse slides in 1x TBST
- 30 minutes at Room Temp: Apply a biotinylated secondary antibody (specific to the host of the primary antibody)

- 1 minute at Room Temp: Rinse slides in 1x TBST
- 30 minutes at Room Temp: Apply alkaline phosphatase streptavidin
- 1 minute at Room Temp: Rinse slides in 1x TBST
- 30 minutes at Room Temp: Apply alkaline phosphatase chromogen substrate
- 1 minute at Room Temp: Wash slides with distilled water

Dehydration: At Room Temperature

- Chromogen substrate has to be alcohol insoluble for this method to work!
- 1 minute each: Wash slides in 2 changes of 80% alcohol
- 1 minute each: Wash slides in 2 changes of 95% alcohol
- 1 minute each: Wash slides in 2 changes of 100% alcohol
- 1 minute each: Wash slides in 3 changes of xylene
- Apply coverslip