

The IHC-plus™ Immunohistochemistry Protocol

Tissue Preparation

Formalin fixation and embedding in paraffin wax

Tissue Sectioning

Make 4- μ m sections and place on pre-cleaned and charged microscope slides.
Heat in a tissue-drying oven for 45 minutes at 60°C

Deparaffinization

Wash slides in 3 changes of xylene – 5 minutes each at room temperature.

Rehydration

Wash slides in 3 changes of 100% alcohol – 3 minutes each at room temperature.
Wash slides in 2 changes of 95% alcohol – 3 minutes each at room temperature.
Wash slides in 1 change of 80% alcohol – 3 minutes at room temperature.
Rinse slides in gentle running distilled water – 5 minutes at room temperature.

Antigen retrieval

Steam slides in 0.01 M sodium citrate buffer, pH 6.0 at 99-100°C - 20 minutes
Remove from heat and let stand at room temperature in buffer - 20 minutes
Rinse in 1X TBS with Tween (TBST) – 1 minute at room temperature.

Immunostaining

Do not allow tissues to dry at any time during the staining procedure.
Apply a universal protein block – 20 minutes at room temperature.
Drain protein block from slides, apply diluted primary antibody – 45 minutes at room temperature.
Rinse slides in 1X TBST - 1 minute at room temperature.
Apply a biotinylated secondary antibody (specific to the host of the primary antibody) - 30 minutes at room temperature.
Rinse slides 1X TBST – 1 minute at room temperature.
Apply alkaline phosphatase streptavidin – 30 minutes at room temperature.
Rinse slides in 1X TBST - 1 minute at room temperature.
Apply alkaline phosphatase chromogen substrate – 30 minutes at room temperature.
Wash slides in distilled water – 1 minute at room temperature.

Suggested reagents used:

Vector ABC-AP (AK-5000) reagent, followed by a Vector Red (SK-5100) substrate. Finally, stain with Hematoxylin alone (no Eosin).

Dehydrate

This method should only be used if the chromogen substrate is alcohol insoluble.
Wash slides in 2 changes of 80% alcohol – 1 minute each at room temperature.
Wash slides in 2 changes of 95% alcohol – 1 minute each at room temperature.
Wash slides in 3 changes of 100% alcohol – 1 minute each at room temperature.
Wash slides in 3 changes of xylene – 1 minute each at room temperature.
Apply coverslip

