

Protocol**Anti-Collagen Immunohistochemistry (IHC)****I. Reagents Required**

Product	Preparation	Item No.
1X TBS with Tween	Use 10X TTBS, pH 7.5. Dilute appropriate volume to 1X with deionized water	MB-013
Xylene	(e.g. PolyMount Mounting Media MaxTag™ Histo for IHC)	KHH001
80%, 95%, and 100% Ethanol		
UltraPure Sterile Water	Make 1X solution by diluting with UltraPure Sterile Water	MB-009-1000
Primary Antibody		
0.01M Sodium Citrate Buffer, pH6.0		
Universal Protein Block	(e.g., Normal Goat Serum (NGS) (#B304) if secondary antibody is goat host)	
Biotinylated Secondary Antibody		
Alkaline Phosphatase Chromogen Substrate		

II. Tissue Preparation and Sectioning

1. Formalin fixation and embedding in paraffin wax.
2. Make 4 µm sections and place on pre-cleaned and charged microscope slides.
3. Heat in a tissue-drying oven for 45 minutes at 60°C.

III. Procedure for Paraffin Sections

1. Deparaffinize slides in xylene 3 times for 5 minutes each at room temperature.
2. Hydrate slides with 100% ethanol 3 times for 3 minutes each at room temperature.
3. Hydrate slides with 95% ethanol 2 times for 3 minutes each at room temperature.
4. Hydrate slides with 80% ethanol 1 time for 3 minutes at room temperature.
5. Rinse in UltraPure sterile water for 5 minutes at room temperature.

IV. Antigen Retrieval

1. Steam slides in 0.01M sodium citrate buffer, pH 6.0 at 99–100°C for 20 minutes.
2. Remove from heat and let stand at room temperature in buffer for 20 minutes.

3. Rinse in 1X TBS with Tween (TBST) for 1 minute at room temperature.

V. Immunostaining

1. Do not allow tissues to dry at any time during the staining procedure.
2. Apply a universal protein block for 20 minutes at room temperature.
3. Drain protein block from slides, apply diluted primary antibody for 45 minutes at room temperature.
4. Rinse slides in 1X TBST for 1 minute at room temperature.
5. Apply biotinylation secondary antibody (specific to the host of primary antibody) for 30 minutes at room temperature.
6. Rinse slides in 1X TBST for 1 minute at room temperature.
7. Apply alkaline phosphatase chromogen substrate for 30 minutes at room temperature.
8. Rinse in UltraPure sterile water for 1 minute at room temperature.

VI. Dehydrate

1. This method should only be used if the chromogen substrate is alcohol insoluble.
2. Wash slides with 80% ethanol 2 times for 1 minute each at room temperature.
3. Wash slides with 95% ethanol 2 times for 1 minute each at room temperature.
4. Wash slides with 100% ethanol 3 times for 1 minute each at room temperature.
5. Wash slides with xylene 3 times for 1 minute each at room temperature.
6. Apply coverslip.

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