

Anti-Collagen Immunohistochemistry (IHC) Protocol

I. Reagents and Equipment Required

Reagent/Equipment

1X TBS with Tween (10X TBST - #MB-013)

Xylene (PolyMount Mounting Media MaxTag™ Histo for IHC - #KHH001)

80%, 95%, and 100% Ethanol

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.

delivery. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Immunochemicals, Inc., P.O. BOX 5199, Limerick, Pennsylvania 19468, USA.

© 2022 Rockland Immunochemicals, Inc. All rights reserved.