Tissue Immunostaining Protocol

Rat Antibodies to detect alpha-chains of type IV collagen NC1

Catalog #7070 (H11), 7071 (H22), 7072 (M69), 7073 (H43), 7074 (H63), 7076 (H31), 7077 (H52) and 7079 (M54)

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A. CRYOSTAT SECTIONS

An immunostaining method to detect alpha-chains of type IV collagen NC1 in cryostat sections by rat monoclonal antibodies. The following protocol may need to be optimized depending on sample type.

1. Cut 3 µm thick cryostat sections, air dry, and fix in acetone for 10 minutes at room temperature.
2. Wash the slides in 0.05M phosphate saline buffer, pH 7.4 for 5 minutes.
3. Incubate the sections in 0.1M glycine/6M urea solution, pH 3.2 for 30 minutes at room temperature.
4. Rinse the slides in 0.05M phosphate saline buffer, pH 7.4. for 2 minutes.
5. Block the slides with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 30 minutes at room temperature.
6. Incubate the sections with a monoclonal antibody diluted 1:50 with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour at room temperature.
7. Wash the slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes.
8. Incubate the sections with FITC-labeled anti-rat IgG antibodies in 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour at room temperature.
9. Wash the slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes and mount the slides with a media containing p-Phenylenediamine to delay fluorescence quenching.

B. PARAFFIN-EMBEDDED TISSUE SECTIONS

An immunostaining method to detect alpha-chains of type IV collagen NC1 in formalin-fixed, paraffin-embedded tissue sections by rat monoclonal antibodies. The following protocol may need to be optimized depending on sample type.

1. Cut 3-4 µm thick formalin-fixed, paraffin-embedded tissue sections, deparaffinize, and rehydrate.
2. Immerse the sections with 0.2M HCl, pH 0.9 and heat in a small autoclave at 110-127°C for 6 minutes. (Alternatively, boil sections in citrate buffer, pH 2.0 in a microwave oven at 750W for 8 minutes followed by 350W for 15 minutes)
3. Rinse the slides in distilled water for 2 minutes.
4. Incubate the sections in 0.1M glycine/6M urea solution, pH 3.2 for 30 minutes at room temperature.
5. Rinse the slides in 0.05M phosphate saline buffer, pH 7.4. for 2 minutes.
6. Block the sections with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 30 minutes at room temperature.
7. Incubate the sections with a monoclonal antibody diluted 1:100 with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour at room temperature.
8. Wash the slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes.
9. Incubate the sections with FITC-labeled anti-rat IgG antibodies in 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour at room temperature.
10. Wash the slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes and mount the slides with a media containing p-Phenylenediamine to delay fluorescence quenching.

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