Collagen Type 1α1

Abcam (Cat # ab90395)

Immunohistochemistry Protocol For Formalin Fixed Paraffin Embedded Tissue
Cut sections at 3 microns and bake overnight at 60°C

Day 1

1. Bake slides at 60°C for minimum of 30 minutes prior to staining.

2. Deparaffinize tissues in xylene (3 changes for 5 minutes each), and rehydrate through 2 changes in 100% Ethanol, 2 changes in 95% Ethanol and 1 change in 70% Ethanol for 5 minutes each).

3. Wash twice in deionized water for 5 minutes each.

4. Perform antigen retrieval with Proteinase K (Invitrogen 25530-015) at room temperature for 10 min.

5. Rinse the slides in 3 changes of deionized water.

6. Outline each section with a PAP pen.

7. Quench endogenous peroxidase activity with DAKO dual endogenous enzyme blocking reagent (Dako S2003) for 30 min.

8. Rinse the slides in 2 changes of deionized water.

9. Prepare the primary antibody in 1X PBS. Negative control slides can be incubated with 1X PBS.

10. Incubate the slides with a 1:2000 dilution of Collagen type 1α1 primary antibody (Abcam cat# ab90395) overnight at 4°C
DAY 2

1. Rinse slides in 1X PBST - 3 times for 5 minutes each.

2. Incubate with Rabbit Antibody Amplifier from MaxPoly-Two Polymer HRP rabbit detection kit (Maxvision Bio PT03-L) for 15 minutes.

3. Rinse slides in 1X PBST - 3 times for 5 minutes each.

4. Incubate slides with Polymer HRP from MaxPoly-Two Polymer HRP rabbit detection kit (Maxvision Bio PT03-L) for 15 minutes.

5. Rinse slides in 1X PBST - 3 times for 5 minutes each.

6. Detect color reaction with Vector Immpact DAB (Vector SK-4105) for a few minutes (check under microscope) until staining intensity is optimal.

7. Stop the reaction with deionized water.

8. Counterstain the sections with Hematoxylin (Zymed Cat # 93-3943) for 5 minutes.

9. Rinse in tap water.

10. Place slides in 1X PBS for 5 minutes.

11. Rinse with deionized water.

12. Dehydrate quickly through 3 changes of 95% ethanol and 2 changes of 100% ethanol.

13. Clear in 3 changes of xylene and mount with Cytoseal.

Standardized on Mouse tissue on 12/19/2011 by Ashish Thomas, M.S.