



Collagen Type 3a1

Abcam cat# ab7778

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 30 minutes** prior to starting IHC
2. Deparaffinize slides in xylenes for 5min each, and rehydrate through graded alcohols (100% EtOH twice 95% EtOH twice and 70% EtOH for 5 min each)
3. Wash twice in deionized water for 5 min each.
4. Perform antigen retrieval using 10µg/ml Proteinase K at room temperature.
5. Incubate slides **at room temperature for 10 minutes.**
6. Rinse off enzyme solution in deionized water.
7. Outline each section with a **PAP pen.**
8. Quench endogenous peroxidase in **DAKO Endogenous Blocking Reagent (Dako S2003) for 30 min.**
9. Rinse thrice with deionized water, then once in 1X PBST.
10. Block non-specific binding sites with **the diluted Vectastain Normal Goat Serum (Vectastain Elite Rabbit Kit PK-6101) – incubate for 30 min.**
11. **Do not** rinse the serum off after the incubation period.
12. Prepare primary Ab in **2% Normal Goat Serum.**
13. Drain and incubate **overnight at 4°C** with a **1:1000 dilution of Collagen 3a1 primary antibody**
(Abcam ab7778)



DAY 2

1. Let slides warm up to room temperature for 30 minutes, then wash 5 times with 1X PBST for 5 min each.
2. Incubate with **Vectastain Biotinylated Anti-Rabbit secondary antibody** (*Vectastain Elite Universal Kit PK-6101*) for **30 minutes**.
3. Reconstitute the **Vectastain ABC reagent** (*Vectastain Elite Universal Kit PK-6101*) and incubate at **room temperature for 30 min**.
4. Wash 5 times with 1X PBST for 5 min each.
5. Incubate the sections with **Vectastain ABC reagent** for **30 minutes**.
6. Wash well with 4 changes of 1X PBST, followed by 2 changes of deionized water
7. Detect color with **Vector Impact DAB** (*Vector SK-4105*) for **30sec to 1min** (check under the microscope)
8. Stop the color reaction with deionized water.
9. Counterstain the sections in **Hematoxylin** (Invitrogen Cat # 00-8001) for 5 minutes.
10. Rinse with tap water.
11. Place the slides in 1X PBS for 1-3 minutes.
12. Rinse well with deionized water.
13. Dehydrate through 2 changes of 95% ethanol and 2 changes of 100% ethanol
14. Clear in 3 changes of Xylene - mount with Cytoseal.

Evidence of positive staining

Collagen type 3a1 occurs in most soft connective tissues along with Collagen type 1.

Protocol standardized on mouse tissue on 4/30/2013 by Ashish Thomas, M.S.