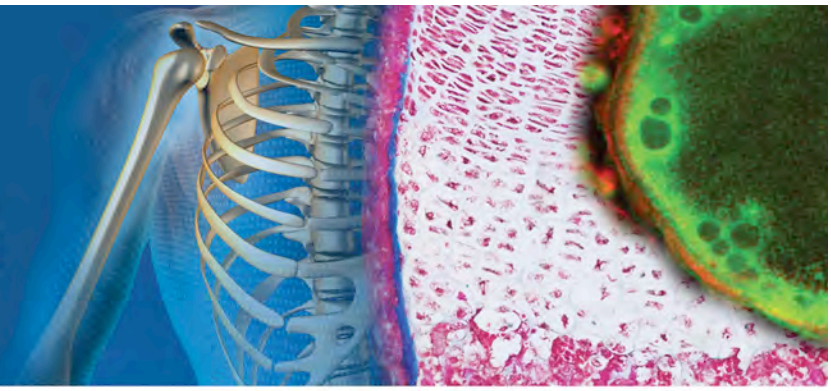

Collagen Type 10a1

Quartett Cat # 2031501005.
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. Enzymatic Antigen Retrieval: Perform antigen retrieval using **Pepsin** *(Sigma P-7000)*
5. (See Pepsin Digest in notes below)
6. Incubate the slides **at 37°C** in a water bath **for 10 minutes.**
7. Rinse off pepsin solution in deionized water.
8. Outline each section with a **PAP pen.**
9. Quench endogenous peroxidase in **DAKO Endogenous Blocking Reagent (***Dako S2003***) for 30 min.**
10. Rinse thrice with deionized water then once in 1X PBST.
11. Block non-specific binding sites with **the diluted Vectastain Normal Horse Serum (***Vectastain Elite Universal Kit PK-6200***) – incubate for 30 min.**
12. **Do not** rinse the serum off after the incubation period.
13. Drain and incubate **overnight at 4°C** with a **1:100 dilution of Collagen X primary antibody** *(Quartett Cat # 2031501005*).
14. Use a **1:200 dilution for embryo sections**. Prepare primary antibody **in 2% Normal Horse Serum**. Negative Control slides need to be incubated with **2% Normal Horse Serum** only**.**

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 Horse Anti-Universal secondary antibody (***Vectastain Elite Universal Kit PK-6200***) for 30 minutes.**
3. Reconstitute the **Vectastain ABC reagent (***Vectastain Elite Universal Kit PK-6200***)** and incubate at **room temperature for 30 min.**
4. Wash 5 times with 1X PBST (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 Immpact DAB** *(Vector SK-4105)* **for a few minutes** (check under the microscope)
8. Stop the color reaction with deionized water.
9. Counterstain the sections with **Hematoxylin (***Zymed Cat # 93-3943***)** for 5 minutes.
10. Rinse with tap water
11. Place slides in 1X PBS for 1-3 minutes.
12. Rinse 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.

¶ **Buffers:-**

0.01 N HCl:

1000 ml of deionized H2O + 900 μl of concentrated (12N) HCl

Pepsin Digest:

Dissolve 1 gm of Pepsin powder *(Sigma P-7000)* in 250 ml of 0.01 N HCl solution pre-heated to 37ºC. Add the entire solution into a plastic stain bucket. Place the slides in a slide rack and slowly immerse the rack into the Pepsin solution. Cover the stain bucket with its lid and place in 37ºC water bath for 10 minutes.

Vectastain Elite Universal IgG Kit (PK-6200):

1X PBST can be used as the diluent buffer for the blocking serum and secondary antibody. Follow the instructions written on the kit data sheet.

¶ Evidence of positive staining:-

Collagen type X expression should be localized in the hypertrophic zones of the Growth Plate, as well as the stromal osteoblastic cell populations within the trabecular bone.

Revised on 10/28/2010 by Ashish Thomas, M.S.