



Collagen Type 2a1

Thermo Scientific Catalog # MS235-P

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. (See notes below)
5. Incubate slides at **37°C** in a water bath **for 10 minutes**.
6. Rinse off pepsin 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 Horse Serum (Vectastain Elite Universal Kit PK-6200) – incubate for 30 min**.
11. **Do not** rinse the serum off after the incubation period.
12. Prepare primary Ab in **2% Normal Horse Serum**.
13. Drain and incubate **overnight at 4°C** with a **1:100 dilution of Collagen II primary antibody (Thermo Scientific Catalog # MS235-P)**.
14. Use a **1:200 dilution for embryo (E18.5) sections**.
15. 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 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 a few minutes** (check under the microscope)
8. Stop the color reaction with deionized water.
9. Counterstain the sections in **Hematoxylin** (*Zymed Cat # 93-3943*) 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.

Pepsin Digest:

0.01 N HCl:

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

Dissolve 1 gm of Pepsin powder (Sigma P-7000) in 250 ml of 0.01 N HCl solution pre-heated to 37°C. 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 37°C 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, secondary antibody and ABC reagent. Follow the instructions written on the kit data sheet.

¶ **Evidence of positive staining:-**

Collagen type II expression should be localized in the hypertrophic zones of the Growth Plate and in the Articular Cartilage; as well as the stromal osteoblastic cell populations within the trabecular bone.

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