



Aggrecan

Chemicon (AB1031)

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 5 min each and rehydrate through graded alcohols (100% - 70% EtOH for 5 min each)
3. Wash twice in deionized water for 5 min each.
4. Enzymatic Antigen Retrieval – Perform antigen retrieval using **Hyaluronidase** (*Sigma H3506*) in 1X PBS.
5. Incubate slides at 37°C in a water bath for 10 minutes.
6. Rinse the slides in deionized water.
7. Outline each section with a PAP pen.
8. Quench endogenous peroxidase in **DAKO Endogenous Blocking Reagent for 30 min** (*Dako S2003*)
8. Rinse the slides in 2 changes of deionized water and once in 1X PBST.
9. Block non-specific binding sites **the diluted Vectastain Normal Goat Serum** (*Vectastain Elite Rabbit IgG Kit PK-6101*) – **incubate for 30 min.**
Do not rinse slides after the incubation period.
10. Block endogenous avidin in the tissue with **Vector Avidin reagent.** (*Vector SP-2001*) for 15 minutes.
11. Rinse twice with 1X PBST.
12. Block endogenous biotin in the tissue with **Vector Biotin reagent.** (*Vector SP-2001*) for 15 minutes.
13. Rinse twice with 1X PBST.
14. Prepare the primary antibody in **2% Normal Goat Serum**, this decreases non-specific staining. Negative control slides need to be incubated with 2% normal goat serum only.
15. Incubate the slides with a **1:200 dilution of Aggrecan primary antibody** (*Chemicon AB1031*) **overnight at 4°C**



1. Let slides warm up to room temperature for 15-30 minutes.
2. Wash the slides 5 times with 1X PBST for 5 minutes each.
3. Incubate with **Vectastain Biotinylated Goat anti-rabbit** secondary antibody (*Vectastain Elite Rabbit IgG Kit PK-6101*) for **30 min.**
4. 4. Wash the slides 5 times with 1X PBST for 5 minutes each.
5. Reconstitute the **Vectastain ABC reagent** and incubate at **room temperature for 30 min.**
6. Incubate with **Vectastain ABC reagent** for **30 min.**
7. Wash the slides 4 times with 1X PBST, then twice in deionized water.
8. Detect color reaction with **Vector Impact DAB** (*Vector SK-4105*) for a few minutes (check under microscope) until staining intensity is optimal.
9. Stop the reaction with deionized water.
10. Counterstain the sections with **Hematoxylin** (*Zymed Cat # 93-3943*) for 5 minutes.
11. Rinse in tap water.
12. Place slides in 1X PBS for 1-3 minutes.
13. Rinse with deionized water.
14. Dehydrate quickly through 3 changes of 95% ethanol and 2 changes of 100% ethanol.
15. Clear in 3 changes of xylene and mount with Cytoseal.

Vectastain Elite Rabbit IgG Kit (PK-6101)

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:

Expression should be localized to the growth plate and articular chondrocytes.

(Mouse tissue protocol standardized on 3/29/2012 by Ashish Thomas, M.S.)