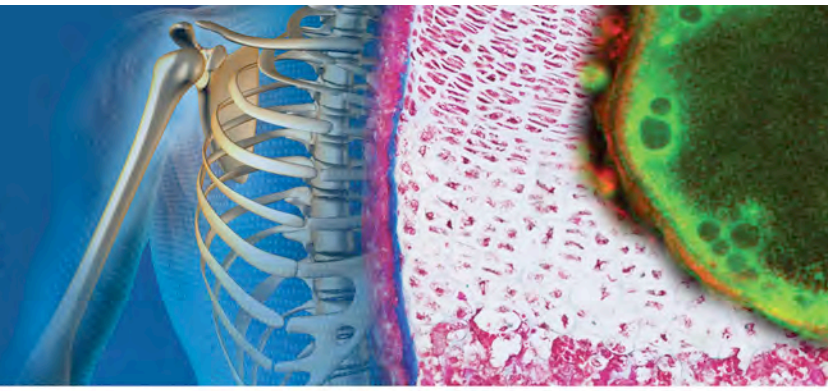

Aggrecan (NITEGE)

MDBioProducts cat#1042003
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.
2. Deparaffinize slides in xylenes for 5min each and rehydrate through graded alcohols (100% - 70% EtOH for 5 min each)
3. Wash 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)*
9. Rinse thrice with deionized water, then once in 1X PBST.
10. Block non-specific binding sites with the diluted **Vector Normal Horse Serum** *(Vectastain Elite Mouse Kit PK-6102).* – incubate for **30 min.**
11. Drain off the normal serum. Do not rinse or wash it off the slides.
12. Block non-specific binding sites with the **BEAT Blocking Solutions** *(Invitrogen cat# 50-300*) Add 2 drops of blocking solution **1A** to each section and incubate for 30 min.
13. Rinse the sections in deionized water.
14. Then rinse the sections twice in 1X PBST for 3 minutes each.
15. Add 2 drops of blocking solution **1B** to each section and incubate for 10 min.
16. Rinse the sections in deionized water.
17. Then rinse the sections twice in 1X PBST for 3 minutes each.
18. Drain and incubate overnight at 4°C with **a 1:400** dilution of **NITEGE primary antibody** *(MDBioProducts cat#1042003)* for 2-3 month old adult sections.
19. Prepare the primary antibody in 2% Normal Horse Serum *(Vectastain Elite Mouse Kit PK-6102).* Negative Control slides need to be incubated with 2% Normal Horse Serum only.

DAY 2

1. Let slides warm up to room temperature, then wash 5 times with 1X PBST for 5 min each**.**
2. Incubate the sections with **Vectastain Biotinylated Horse Anti-mouse secondary antibody (***Vectastain Elite Mouse Kit PK-6102***) for 30 minutes.**
3. Reconstitute the **Vectastain ABC reagent (***Vectastain Elite Mouse Kit PK-6102***)** 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 the slides 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 distilled water.
13. Dehydrate through 2 changes of 95% ethanol and 2 changes of 100% ethanol.
14. Clear in 3 changes of Xylene and then mount with Cytoseal.

¶ Notes-

Hyaluronidase Digest:

Dissolve 25mg of Hyaluronidase obtained from bovine testes *(Sigma Cat # H3506-1G)* in 250 ml of 1X PBS solution pre-heated to 37ºC. Add the entire solution into a stain bucket. Place the slides in a slide rack and slowly immerse the rack into the warm Hyaluronidase solution. Cover the stain bucket with its lid and place in 37ºC water bath for 10 minutes.

Vectastain Elite Mouse IgG Kit (PK-6102):

1:20 BSA-PBST can be used as the diluent buffer for the blocking serum and secondary antibodies. Follow the instructions written on the kit data sheet.

¶ Evidence of positive staining:-

Expression should be localized to the hypertrophic zones of the Growth plate. Articular Cartilage chondrocytes are **negative** for NITEGE in wild type sections.

Revised on 7/15/11 by Ashish Thomas, M.S