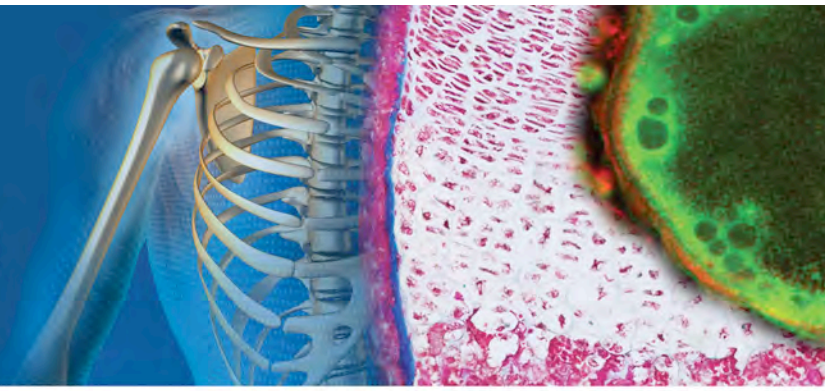

Aggrecan (DIPEN)

MDBioProducts cat#1042002 (Mouse Monoclonal Antibody)

Immunohistochemistry Protocol for EDTA 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. No Antigen Retrieval required.
5. Outline each section with a **PAP pen.**
6. Quench endogenous peroxidase in **DAKO Endogenous Peroxide Blocking Reagent for 30 min (***Dako S2003***)**
7. Rinse thrice with deionized water, then once in 1X PBST.
8. Block non-specific binding sites **with 1:20 Vector Normal Horse Serum (***Vector S-2000***). Incubate the slides for 30 min.**
9. Drain off the normal serum. Do not rinse or wash it off the slides.
10. Block non-specific binding sites **with the BEAT Blocking Solutions (***Invitrogen 50-300)* Add 2 drops of blocking solution **1A** to each section and **incubate for 30 min.**
11. Rinse the sections in deionized water.
12. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
13. Add 2 drops of blocking solution **1B** to each section and **incubate for 10 min.**
14. Rinse the sections in deionized water.
15. Then rinse the sections **twice** in 1X PBST **for 3 minutes each.**
16. Drain and incubate **overnight at 4°C** with a **1:800** dilution of DIPEN primary antibody (*MDBioProducts cat#1042002)* for **2-3 month old adult sections.**
17. Prepare the primary antibody **in 1:50 Normal Horse Serum (***Vector S-2000***)**. 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 **1:1000 of Biotinylated Horse Anti-mouse secondary antibody (***Vector BA-2000***) for 30 minutes.**
3. Wash 5 times with 1X PBST for 5 min each.
4. Incubate the sections with **1:1000 of Streptavidin-HRP reagent (***Thermo Scientific 21130)* for **30 minutes.**
5. Wash the slides well with 4 changes of 1X PBST, followed by 2 changes of deionized water
6. Detect color with **Vector Immpact DAB** *(Vector SK-4105)* **for a few minutes** (check under the microscope)
7. Stop the color reaction with deionized water.
8. Counterstain the sections with **Hematoxylin (***Zymed Cat # 93-3943***)** for 5 minutes.
9. Rinse with tap water
10. Place slides in 1X PBS for 1-3 minutes.
11. Rinse with distilled water.
12. Dehydrate through 2 changes of 95% ethanol and 2 changes of 100% ethanol.
13. Clear in 3 changes of Xylene and then mount with Cytoseal.

¶ Evidence of positive staining:-

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

(Revised on 8/10/11 by Ashish Thomas)