



### PRG-4 (Lubricin)

Abcam cat # ab28484.

Immunohistochemistry Protocol For Formalin Fixed Paraffin Embedded Tissues.

Cut sections at 5 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% - 70% EtOH for 5 min each)
3. Wash twice in deionized water twice for 5 min each.
4. Enzymatic Antigen Retrieval: Perform antigen retrieval using **Hyaluronidase** (*Sigma H3506*) Incubate slides at **37°C** in a water bath **for 10 minutes**.
5. See Hyaluronidase Digest in notes below.
6. Rinse the slides 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 the slides thrice with deionized water, then once in 1X PBST.
10. Block non-specific binding sites with **the diluted Vectastain Normal Goat Serum** (*Vectastain Elite Rabbit IgG Kit PK-6101*) – **incubate for 30 min**.
11. Do **NOT** rinse the serum off after the incubation period.
12. Drain the serum and incubate **overnight at 4°C** with a **1:400** dilution of PRG-4 primary antibody (*Abcam cat # ab28484*) for **5-6 month old adult sections**.
13. Prepare primary Ab in **2% Normal Goat Serum**.
14. Negative Control slides need to be incubated with **2% Normal Goat Serum** only.



## DAY 2

1. Let slides warm up to room temperature then rinse 5 times with 1X PBST for 5 minutes each.
2. Incubate with **Vectastain Biotinylated Goat Anti-Rabbit secondary antibody** (*Vectastain Elite Rabbit IgG Kit PK-6101*) for **30 minutes**.
3. Reconstitute the **Vectastain ABC reagent** and incubate at **room temperature for 30 min** (*Vectastain Elite Rabbit IgG Kit PK-6101*).
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)
17. Stop the color reaction with deionized water.
18. Counterstain the sections with **Hematoxylin** (*Zymed Cat # 93-3943*) for 5 minutes.
19. Rinse with tap water.
20. Place the slides in 1X PBS for 1-3 minutes.
21. Rinse with deionized water.
22. Dehydrate through 2 changes of 95% ethanol and 2 changes of 100% ethanol
23. Clear in 3 changes of Xylene and then mount with Cytoseal.

### **¶ 0.01 % Hyaluronidase Digest:**

Dissolve 25mg of Hyaluronidase in 250 ml of 1X PBS solution pre-heated to 37°C. Add the hot solution into a plastic stain bucket. Place the slides in a slide rack and slowly immerse the rack into the Hyaluronidase solution. Cover the bucket and place in 37°C water bath for 10 minutes.

### **¶ 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:-**

Articular Cartilage chondrocytes are positive for PRG-4.

Protocol standardized on mouse tissue on 3/8/11 by Ashish Thomas, M.S.