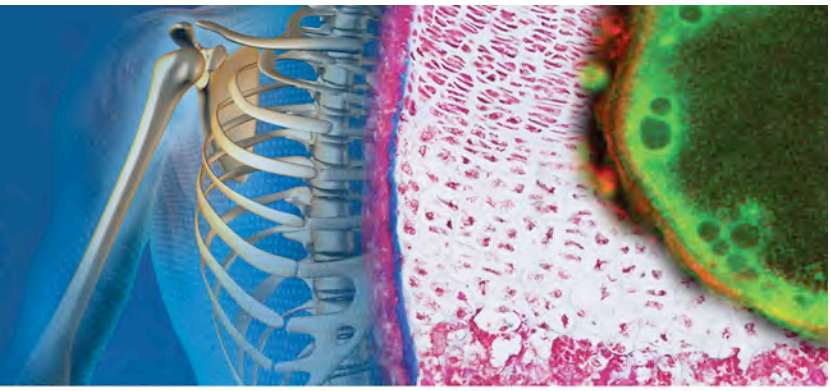

Tak1 (Total)

Santacruz cat# sc7162
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 in deionized water for 5 min each.
4. Enzymatic Antigen Retrieval – Perform antigen retrieval using **Hyaluronidase** *(Sigma H-3506)* 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 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 Vectastain Normal Goat Serum (***Vectastain Elite Rabbit Kit PK-6101***) – incubate for 30 min.**
11. Do **NOT** rinse the serum off after the incubation period.
12. Drain and incubate **overnight at 4°C** with a **1:50** dilution of **Tak1 primary antibody** (*Santacruz cat# sc7162)* for **2-3 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 wash 5 times with 1X PBST for 5 min each**.**
2. Incubate the sections with **Vectastain Biotinylated Goat Anti-Rabbit secondary antibody (***Vectastain Elite Rabbit Kit PK-6101***) for 30 minutes.**
3. Reconstitute the **Vectastain ABC reagent (***Vectastain Elite Rabbit Kit PK-6101***)** 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 DAB Immpact** *(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.

¶ Buffers:-

1X PBST

Dilute 10 X PBS to 1X with deionized water.
Add Tween-20 to produce a final concentration of 0.01% Tween in 1X PBS.

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 Rabbit IgG Kit (PK-6101):

1:20 PBST-BSA 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 Tak1.

Protocol standardized on mouse tissue on 4/15/11 by Ashish Thomas, M.S.