



P27

Santacruz Biotech cat# sc528.
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 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*) Incubate slides at **37°C** in a water bath **for 10 minutes**. See Hyaluronidase Digest in notes below.
5. Rinse the slides in deionized water.
6. Outline each section with a **PAP pen**.
7. Quench endogenous peroxidase in **DAKO Endogenous Blocking Reagent for 30 min** (*Dako S2003*)
8. Rinse thrice with deionized water, then once in 1X PBST.
9. Block non-specific binding sites with **the diluted Vectastain Normal Goat Serum** (*Vectastain Elite Rabbit IgG Kit PK-6101*) – **incubate for 30 min**.
10. Do **NOT** rinse the serum off after the incubation period.
11. Drain and incubate **overnight at 4°C** with a **1:200** dilution of p27 primary antibody (*Santacruz Biotech # sc528*) for **2-3 month old adult sections**.
12. Prepare primary Ab in **2% Normal Goat Serum**.
13. Use a **1:400 dilution** for **embryo (E18.5) sections**.
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 (5 min each)
2. Incubate the sections with **Vectastain Biotinylated Goat Anti-Rabbit secondary antibody** (*Vectastain Elite Rabbit IgG Kit PK-6101*) for **30 minutes**
3. Reconstitute the **Vectastain ABC reagent** (*Vectastain Elite Rabbit IgG 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 Impact** (*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 in 250 ml of 1X PBS solution pre-heated to 37°C. Add the entire solution into a stain bucket. Place the slides in a slide rack and slowly immerse the rack into the Hyaluronidase solution. Cover the stain bucket with its lid 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:-**

p27 expression should mostly be localized to the pre-hypertrophic chondrocytes of the Growth plate.

Protocol standardized on mouse tissue on 2/25/11 by Ashish Thomas, M.S.