



## Sox-9

### Santacruz (sc 20095)

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 minimum of 30 minutes** prior to staining.
2. Deparaffinize tissues in xylene (3 changes for 5 minutes each), and rehydrate through 2 changes in 100% Ethanol, 2 changes in 95% Ethanol and 1 change in 70% Ethanol for 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Perform antigen retrieval using **Proteinase K** (*Invitrogen 25530-015*) at **37°C for 10 min.**
5. Rinse the slides in 3 changes of deionized water.
6. Outline each section with a **PAP pen.**
7. Quench endogenous peroxidase activity with **DAKO dual endogenous enzyme blocking reagent** (*Dako S2003*) **for 30 min.**
8. Rinse the slides in 2 changes of deionized water.
9. Block non-specific binding sites with diluted **Vectastain Normal Goat Serum** (*Vectastain Elite Rabbit IgG Kit PK-6101*) **for 30 minutes.** Prepare the primary antibody in **Normal Goat Serum.**
10. Drain serum off the slides and incubate them with a **1:50 to 1:100 dilution of Sox-9 primary antibody** (*Santacruz cat# sc20095*) for E18.5 embryo sections **overnight at 4°C**



## Day 2

1. Let slides warm up to room temperature for 15-30 minutes.
2. Wash the slides 5 times with 1X PBST for 5 minutes each.
3. Incubate with **Vectastain Biotinylated Goat anti-rabbit** secondary antibody (*Vectastain Elite Rabbit IgG Kit PK-6101*) for **30 min.**
4. 4. Wash the slides 5 times with 1X PBST for 5 minutes each.
5. Reconstitute the **Vectastain ABC reagent** and incubate at **room temperature for 30 min.**
6. Incubate with **Vectastain ABC reagent** for **30 min.**
7. Wash the slides 4 times with 1X PBST, then twice in deionized water.
8. Detect color reaction with **Vector Impact DAB** (*Vector SK-4105*) for a few minutes (check under microscope) until staining intensity is optimal.
9. Stop the reaction with deionized water.
10. Counterstain the sections with **Hematoxylin** (*Zymed Cat # 93-3943*) for 5 minutes.
11. Rinse in tap water.
12. Place slides in 1X PBS for 1-3 minutes.
13. Rinse with deionized water.
14. Dehydrate quickly through 3 changes of 95% ethanol and 2 changes of 100% ethanol.
15. Clear in 3 changes of xylene and mount with Cytoseal.

### **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:**

Sox 9 expression is localized in the chondrocytes.

Protocol standardized on mouse tissue on 7/23/2013 by Ashish Thomas, M.S.