



TRAP

Santacruz (cat# sc30833)
Immunohistochemistry Protocol For Formalin Fixed Paraffin Embedded Tissue
Cut sections at 5 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 graded ethanols (2 changes of 100% ethanol, 2 changes of 95% ethanol and 1 change of 70% ethanol for 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. Perform antigen retrieval with **Sodium Citrate at 65°C for 1 hour in a water bath**. Let the slides cool to room temperature after incubation for 20 minutes.
5. Rinse twice with deionized water.
6. Outline each section with a **PAP pen**.
7. Quench endogenous peroxidase with **DAKO Dual Endogenous Enzyme Blocking Reagent (Dako S2003) for 30 min**.
8. Rinse in 3 changes of deionized water and once in 1X PBST.
9. Block non-specific binding sites **the diluted Vectastain Normal Rabbit Serum (Vectastain Elite Goat IgG Kit PK-6105) – incubate for 30 min**.
10. Block endogenous Avidin receptors in the tissue with **Vector Avidin reagent. (Vector SP-2001) for 15 minutes**.
11. Rinse twice with 1X PBST.
12. Block endogenous Biotin in the tissue with **Vector Biotin reagent. (Vector SP-2001) for 15 minutes**.
13. Rinse twice with 1X PBST.
14. Drain and incubate **overnight at 4°C with a 1:200 dilution of TRAP primary antibody (Santacruz cat# sc30833) for 2-3 month old adult sections**.
15. Prepare the primary antibody in **Normal Rabbit Serum**, this decreases non-specific staining. Negative Control slides need to be incubated with Normal Rabbit Serum only.



Day 2

1. Let slides warm up to room temperature for 30 minutes.
2. Wash the slides 5 times with 1X PBST for 5 minutes each.
3. Incubate with **Vectastain Biotinylated Rabbit anti-goat** secondary antibody (*Vectastain Elite Goat IgG Kit PK-6105*) for **30 min.**
4. Reconstitute the **Vectastain ABC reagent** (*Vectastain Elite Goat IgG Kit PK-6105*) and incubate at **room temperature for 30 min.**
5. Wash 5 times with 1X PBST for 5 minutes each.
6. Incubate with **Vectastain ABC reagent** for **30 min.**
7. Wash 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)
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 95% ethanol (3 changes) and 2 changes of 100% ethanol.
15. Clear in 3 changes of xylene and mount with Cytoseal.

¶ **Vectastain Elite Goat IgG Kit (PK-6105):**

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:**

TRAP expression should be localized to the osteoclasts and bone lining cells.

Protocol standardized on mouse tissue on 8/3/2013 by Ashish Thomas, M.S.