



TNF α

Santacruz cat #sc1350

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, 5 minutes each), and rehydrate through graded alcohols (2 changes 100% and 95%, 1 change 70%, 5 minutes each).
3. Wash twice in deionized water for 5 minutes each.
4. No Antigen Retrieval required.
5. Outline each section with a **PAP pen**.
6. Quench endogenous peroxidase with **DAKO Dual Endogenous Enzyme Blocking Reagent (Dako S2003) for 30 min.**
7. Rinse the slides in 3 changes of deionized water and once in 1X PBST.
8. Block non-specific binding sites **the diluted Vectastain Normal Rabbit Serum (Vectastain Elite Goat IgG Kit PK-6105) - incubate for 30 min.**
9. Drain and incubate **overnight at 4°C with a 1:400 dilution of TNF α primary antibody (Santacruz cat# sc1350) for 2-3 month old adult sections.**
10. Prepare the primary antibody in **2% Normal Rabbit Serum**, this decreases non-specific staining.
11. Negative Control slides need to be incubated with 2% Normal Rabbit Serum only.



Day 2

1. Let slides warm up to room temperature for 30 minutes, then rinse 5 times with 1X PBST for 5 minutes each.
2. Incubate with **Vectastain Biotinylated Rabbit anti-goat** secondary antibody (*Vectastain Elite Goat IgG Kit PK-6105*) for **30 min.**
3. Reconstitute the **Vectastain ABC reagent** (*Vectastain Elite Goat IgG Kit PK-6105*) and incubate at **room temperature for 30 min.**
4. Wash 5 times with 1X PBST for 5 minutes each.
5. Incubate with **Vectastain ABC reagent** for **30 min.**
6. Wash 4 times with 1X PBST, then twice in deionized water.
7. Detect color reaction with **Vector Impact DAB** (*Vector SK-4105*) for a few minutes (check under microscope)
8. Stop the reaction with deionized water.
9. Counterstain the sections with **Hematoxylin** (*Zymed Cat # 93-3943*) for 5 minutes.
10. Rinse in tap water.
11. Place slides in 1X PBS for 1-3 minutes.
12. Rinse with deionized water.
13. Dehydrate quickly through 95% ethanol (3 changes) and 2 changes of 100% ethanol.
14. 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.

Protocol standardized on mouse tissue on 4/15/2011 by Ashish Thomas, M.S.