



RANKL

Santacruz (Cat # sc9073)

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 **10mM Tris EDTA Buffer pH 9.0** – This is done in a water bath at constant temp. **Set water bath to 65°C** and leave slides in the hot water bath for **1 hour**. Then remove cover and leave outside to cool for 10-20 minutes.
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. Prepare the primary antibody in **1X PBS**. Negative control slides can be incubated with 1X PBS.
10. Incubate the slides with a **1:400 dilution of RANKL primary antibody (Santacruz cat# sc9073) overnight at 4°C**



DAY 2

1. Rinse slides in 1X PBST - 3 times for 5 minutes each.
2. Incubate with **Rabbit Antibody Amplifier** from MaxPoly-Two Polymer HRP rabbit detection kit (*Maxvision Bio PT03-L*) for 15 minutes.
3. Rinse slides in 1X PBST - 3 times for 5 minutes each.
4. Incubate slides with **Polymer HRP** from MaxPoly-Two Polymer HRP rabbit detection kit (*Maxvision Bio PT03-L*) for 15 minutes.
5. Rinse slides in 1X PBST - 3 times for 5 minutes each.
6. Detect color reaction with **Vector Impact DAB** (*Vector SK-4105*) for a few minutes (check under microscope) until staining intensity is optimal.
7. Stop the reaction with deionized water.
8. Counterstain the sections with **Hematoxylin** (*Zymed Cat # 93-3943*) for 5 minutes.
9. Rinse in tap water.
10. Place slides in 1X PBS for 5 minutes.
11. Rinse with deionized water.
12. Dehydrate quickly through 3 changes of 95% ethanol and 2 changes of 100% ethanol.
13. Clear in 3 changes of xylene and mount with Cytoseal.

Standardized on Mouse tissue on 8/21/2012 by Ashish Thomas, M.S.