abcam

Product datasheet

Anti-RANKL antibody ab9957

4 Abreviews 14 References 3 Images

Overview

Product name	Anti-RANKL antibody
Description	Rabbit polyclonal to RANKL
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, ELISA, WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Highly pure (>98%) recombinant hsRANK-L (human soluble receptor activator of NF-Kappa B Ligand)
General notes	We have received both positive and negative customer feedback on mouse reactivity for this antibody. Therefore, we do not guarantee this species.

Properties

Form	Lyophilised:Reconstitute with 200µl of sterile water. Please note that if you receive this product in liquid form it has already been reconstituted as described and no further reconstitution is necessary.
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
Storage buffer	PBS, pH 7.4, no preservative, sterile filtered
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	unknown
Light chain type	unknown

Applications

Our Abpromise guarantee covers the use of **ab9957** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★☆	Use a concentration of 1 µg/ml.
ELISA	★★★★☆	Use at an assay dependent dilution. To detect hsRANK-L by direct ELISA (using 100µl/well antibody solution) a concentration of at least 0.5µg/ml of this antibody is required. This antigen affinity purified antibody, in conjunction with compatible secondary reagents, allows the detection of 0.2 - 0.4 ng/well of recombinant hsRANK-L.
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 37 kDa (predicted molecular weight: 35 kDa). To detect hsRANK-L by Western Blot analysis this antibody can be used at a concentration of 0.1 - 0.2 µg/ml. Used in conjunction with compatible secondary reagents the detection limit for recombinant hsRANK-L is 1.5 - 3.0 ng/lane, under either reducing or non-reducing conditions.
IHC-P	★★★★★	Use at an assay dependent dilution.

Target

Function	Cytokine that binds to TNFRSF11B/OPG and to TNFRSF11A/RANK. Osteoclast differentiation and activation factor. Augments the ability of dendritic cells to stimulate naive T-cell proliferation. May be an important regulator of interactions between T-cells and dendritic cells and may play a role in the regulation of the T-cell-dependent immune response. May also play an important role in enhanced bone-resorption in humoral hypercalcemia of malignancy.
Tissue specificity	Highest in the peripheral lymph nodes, weak in spleen, peripheral blood Leukocytes, bone marrow, heart, placenta, skeletal muscle, stomach and thyroid.
Involvement in disease	Defects in TNFSF11 are the cause of osteopetrosis autosomal recessive type 2 (OPTB2) [MIM:259710]; also known as osteoclast-poor osteopetrosis. Osteopetrosis is a rare genetic disease characterized by abnormally dense bone, due to defective resorption of immature bone. The disorder occurs in two forms: a severe autosomal recessive form occurring in utero, infancy, or childhood, and a benign autosomal dominant form occurring in adolescence or adulthood. Autosomal recessive osteopetrosis is usually associated with normal or elevated amount of non-functional osteoclasts. OPTB2 is characterized by paucity of osteoclasts, suggesting a molecular defect in osteoclast development.
Sequence similarities	Belongs to the tumor necrosis factor family.
Post-translational modifications	The soluble form of isoform 1 derives from the membrane form by proteolytic processing (By similarity). The cleavage may be catalyzed by ADAM17.
Cellular localization	Cytoplasm; Secreted and Cell membrane.

Images

Western blot - Anti-RANKL antibody (ab9957)

Anti-RANKL antibody (ab9957) at 1 µg/ml + Human spleen tissue lysate - total protein (ab29699) at 10 µg

Secondary

Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (HRP), pre-adsorbed at 1/3000 dilution

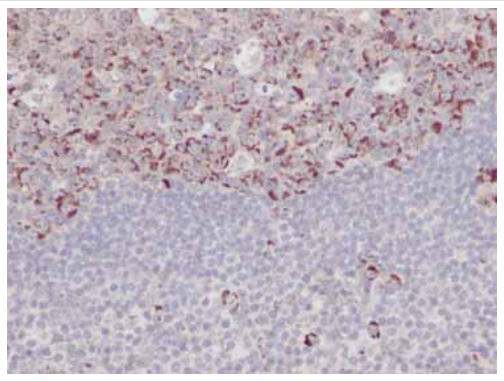
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 35 kDa
Observed band size: 37 kDa
Additional bands at: 72 kDa, 76 kDa. We are unsure as to the identity of these extra bands.

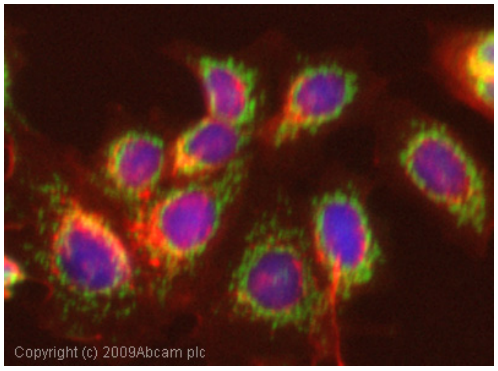
Exposure time: 90 seconds

RANKL contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RANKL antibody (ab9957)

ab9957 staining RANKL in human metastatic carcinoma of lymph nodes from breast cancer tissue by Immunohistochemistry (Formalin/PFA fixed paraffin-embedded sections). Tissue underwent heat mediated antigen retrieval in sodium citrate buffer (pH 6.0). The primary antibody was used at 0.25 ug/ml and incubated with sample at 4°C overnight. A HRP-labeled polymer detection system was used with a DAB chromogen.



Immunocytochemistry/ Immunofluorescence - Anti-RANKL antibody (ab9957)

ICC/IF image of ab9957 stained MCF7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab9957, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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