

Recombinant Human sRANK Ligand Active (Receptor activator of nuclear factor kappa-B ligand)

Human recombinant protein expressed in *Nicotiana benthamiana*

RF0063

Alternative Names: TNFSF11, OPGL, TRANCE

Molecular Formula: C₉₅₂H₁₄₁₄N₂₆₂O₂₇₆S₅

UniProtKB: O14788

p.I: 6.82

Available sizes: 1 µg, 10 µg, 50 µg, 100 µg of active protein

Ext. Coeff. Abs (280nm) 0.1% (=1g/l) =1678

Purity > 97% by SDS-PAGE gel

Endotoxin Level : < 0.04 EU / µg protein (LAL method) □

Molecular Weight:

rhuman sRANKL is a glycosylated polypeptide chain containing 175 amino acids (70 – 244 aa of O14788 TNF11_HUMAN) and a His-tag at the N-terminal end. It has a predicted molecular mass of 21.1 kDa, however as result of glycosylation, the recombinant protein could migrate as two bands with an apparent molecular mass of 21-23 kDa in SDS-PAGE.

Sequence:

HHHHHHHHHHEKAMVDGSLDLAKRSKLEAQPFAHL
TINATDIPSGSHKVSLSWYHDRGWAKISNMFTSNGK
LIVNQDGFYYLYANICFRHHETSGDLATEYLQLMVYVT
KTSIKIPSSHTLMKGGSTKYWSGNSEFHFYSINVGFF
KLRSGEESIEVSNPSLLDPDQDATYFGAFKVRDID

Formulation:

Recombinant human RANKL is lyophilized from 10mM Phosphate Potassium buffer pH 8 and 0.2M NaCl.

Description:

Recombinant human RANKL is a member of TNF super family, a cytokine that play a central role in bone remodeling and disorders of mineral metabolism. It was shown to be a dendritic cell survival factor, T-cell activator and osteoclast regulator because RANKL mediates the osteoclast differentiation, survival and activation. Native RANKL is a type II trans-membrane protein with an extracellular binding domain that interacts with RANK and OPG receptors. OPG protects the skeleton from excessive bone resorption by binding to RANKL and preventing it from binding to its receptor, RANK. Thus, RANKL/OPG ratio became an important determinant of bone mass and skeletal integrity. In addition, this protein was shown to activate anti-apoptotic kinase AKT/PKB through a signaling complex involving SRC kinase and tumor necrosis factor receptor-associated factor (TREAf). Recent findings shown that OPG/RANK/RANKL system has been identifies as a possible mediator of arterial calcification suggesting common links between osteoporosis and vascular diseases.

Source:

Human recombinant protein expressed in *Nicotiana benthamiana*. It is produced by transient expression in non-transgenic plants and is purified by sequential chromatography (FPLC). This product contains no animal-derived components or impurities. Animal Free product.

Reconstitution Recommendation:

Lyophilized protein should be reconstituted in water following instructions of batch Quality Control sheet. At higher concentrations the solubility may be reduced and multimers generated. Optimal concentration should be determined for specific application and cell lines.

Storage and Stability:

This lyophilized preparation is stable at 2-8° C for short term, long storage it should be kept at -20°C. Reconstituted protein should be stored in working aliquots at -20°C. Repeated freezing and thawing is not recommended.

References:

- Anderson, D. et al., 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 390: 175-179
- Takahashi, N. et al., 1999. A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. *Biochem Biophys. Res. Common.* Mar 24; 256(3):449-55.
- Yasuda, H. et al., 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. USA* 95:3597-3602.
- D'Amelio, P. et al., 2009. The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. *J Endocrinol Invest.* 32(4 Suppl):6-9.
- Suda, T. et al., 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand family. *Endocr. Rev.* 20:345-350.

Product(s) expressed through a transient plant system are intrinsically Animal-free

For R+D purposes only. Purchaser must determine the suitability of the product(s) for their particular use.

Applications:

Cell Culture, Western Blot, Immunogen.

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Upon this protein has not been tested in a particular technique this not necessarily excludes its use in such procedures.

Purity Confirmation:

The protein was resolved by SDS polyacrylamide gel electrophoresis and the gel was stained with coomassie blue. Fig. 1, lane 1.

Serological Identification:

The protein was electrophoresed under reducing condition on a 15% SDS-polyacrylamide gel, transferred by electro blotting to a NC membrane and visualized by immune-detection with specific RANKL antibody. Fig. 1, lane 2.

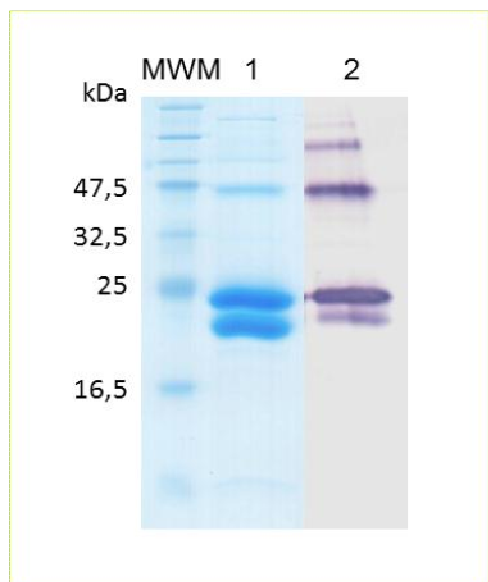


Figure 1.- SDS-PAGE and Western blot analysis of human recombinant sRANKL. Lane MWM: molecular weight marker (kDa). Lane1: coomassie blue 1 µg of recombinant sRANKL and lane 2: 0,1 µg of recombinant sRANKL. All the bands shown in lanes 1-2 have been identify by MALDI-TOFF as recombinant sRANKL.

Biological Activity:

The specific activity is determined by the dose-dependent stimulation of IL-8 production in human PBMC. Activity results may vary with PBMC donors.

Human PBMC stimulated with 10ng/ml of Human recombinant sRANKL increases in three times the basal production of IL-8.

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