Sterile

#5312

Human RANKL/TRANCE/TNFSF11 (hRANKL)

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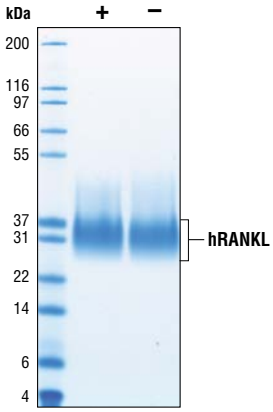
Source: Recombinant human RANKL (hRANKL) Gly63-Asp244 (Accession #NP_143026) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hRANKL contains no "tags" and the nonglycosylated protein has a calculated MW of 20,484. DTT-reduced and non-reduced protein migrate as 30-35 kDa polypeptides. Lower mobility and heterogeneity in SDS-PAGE are due to glycosylation. The expected amino-terminal GSQHI of recombinant hRANKL was verified by amino acid sequencing.

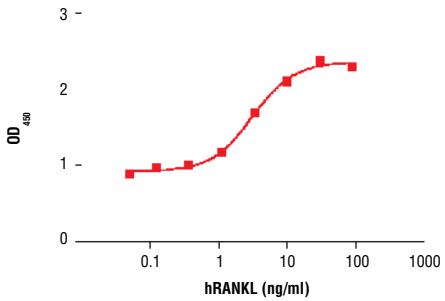
Endotoxin: Less than 0.01 ng endotoxin/1 µg hRANKL.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hRANKL. All lots are greater than 98% pure.



The purity of recombinant hRANKL was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hRANKL and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of hRANKL was determined by measuring the ability of hRANKL to induce TRAP activity in Raw 264.7 cells. The ED_{50} of each lot is between 1.5-5 ng/ml.



The induction of tartrate resistant acid phosphatase (TRAP) in Raw 264.7 cells was assessed. Raw 264.7 cells were treated with increasing concentrations of hRANKL for 4 days. Cells were lysed and TRAP activity of cell extracts was assessed and OD_{450} determined.

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg hRANKL. Carrier free: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 5% sucrose.

Reconstitution:
With carrier: Add sterile PBS or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final hRANKL concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS or PBS containing protein to minimize absorption of hRANKL to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hRANKL should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: RANKL, also known as TRANCE or OPGL, is a member of the TNF superfamily of ligands. T cells, mammary epithelial cells, and endothelial cells can produce RANKL (1). RANKL is expressed as a type II transmembrane protein or cleaved into a soluble form by extracellular proteases, such as TACE, ADAM10, and matrix metalloproteases (1). Alternative splicing also results in the production of soluble RANKL (1). RANKL signaling is antagonized by osteoprotegerin, which functions as a soluble decoy receptor (2). RANKL plays key roles in mammary gland development and dendritic cell survival and is required for osteoclast differentiation and survival (3-6). Research studies have shown that RANKL deficiencies in both mice and humans are associated with abnormally increased bone density and defects in lymphoid organogenesis (5,6).

Background References:

(1) O'Brien, C.A. (2010) *Bone* 46, 911-9.

(2) Lacey, D.L. et al. (1998) *Cell* 93, 165-76.

(3) Wong, B.R. et al. (1997) *J Exp Med* 186, 2075-80.

(4) Fata, J.E. et al. (2000) *Cell* 103, 41-50.

(5) Kong, Y.Y. et al. (1999) *Nature* 397, 315-23.

(6) Conklin, J.L. et al. (1991) *Gastroenterology* 101, 657-63.

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