

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human TRANCE/TNFSF11/RANK L protein		
	Avi-tag	HHHHHH	Human TRANCe/TNFSF11/RANK L (Ile140-Asp317) Accession # O14788.1
	N-terminus		C-terminus
N-terminal Sequence Analysis	Gly of Avi-tag		
Structure / Form	Biotinylated via Avi-tag		
Predicted Molecular Mass	24 kDa		

SPECIFICATIONS

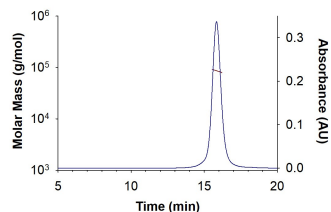
SDS-PAGE	26-39 kDa under reducing conditions.
Activity	Measured by its binding ability in a functional ELISA. When Recombinant Human RANK/TNFRSF11A Fc Chimera Protein (Catalog # 683-RK) is coated at 0.5 µg/mL (100 µL/well), the concentration of Biotinylated Recombinant Human TRANCE/TNFSF11/RANK L His-tag Avi-tag that produces 50% optimal binding response is 3.00-15.0 ng/mL
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

DATA

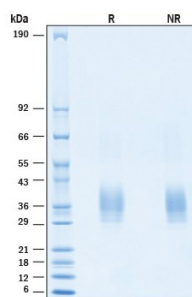
SEC-MALS



SEC-MALS Data	Result
Retention Time	15.5 - 16.2 min
MW - Predicted (Monomer)	24.0 kDa
MW - MALS	85.7 kDa
Polydispersity	1.001
System Suitability:	Pass
BSA Monomer 66.4 ± 3.32 kDa	

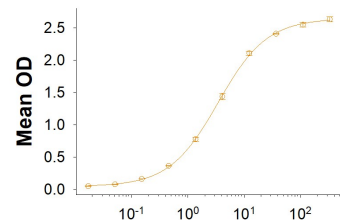
Biotinylated Recombinant Human TRANCF/TNFSF11/RANK L His-tag Avi-tag Protein SEC-MALS. Recombinant Human TRANCF/RANK L/TNFSF11 (Catalog # AVI390) has a molecular weight (MW) of 85.7 kDa as analyzed by SEC-MALS, suggesting that this protein is a homotrimer. MW may differ from predicted MW due to post-translational modifications (PTMs) present (i.e. Glycosylation).

SDS-PAGE



Biotinylated Recombinant Human TRANCF/TNFSF11/RANK L His-tag Avi-tag Protein SDS-PAGE. 2 µg/lane of Biotinylated Recombinant Human TRANCF/TNFSF11/RANK L His-tag Avi-tag Protein (Catalog # AVI390) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 26-39 kDa.

Binding Activity



Biotinylated Recombinant Human TRANCF/TNFSF11/RANK L His-tag Avi-tag (ng/mL)

Biotinylated Recombinant Human TRANCF/RANK L His-tag Avi-tag Protein Binding Activity. When Recombinant Human RANK/TNFRSF11A Fc Chimera Protein (Catalog # 683-RK) is coated at 0.5 µg/mL (100 µL/well), the concentration of Biotinylated Recombinant Human TRANCF/TNFSF11/RANK L His-tag Avi-tag (Catalog # AVI390) that produces 50% optimal binding response is 3.00-15.0 ng/mL.

BACKGROUND

RANK L (receptor activator of NF-kappa B ligand), also called TRANCE (TNF-related activation-induced cytokines), OPGL (osteoprotegerin ligand), or ODF (osteoclast differentiation factor), is a 39-45 kDa type II transmembrane (TM) protein in the tumor necrosis factor family, designated TNFSF11 (1-5). RANK L, produced by osteoblasts and bone marrow stromal cells, is required for differentiation of osteoclasts and stimulates bone resorption (4, 6). It is also produced by activated T cells and augments dendritic cell stimulation; RANK L^{-/-} mice lack lymph nodes and have impaired thymocyte development (1-3, 6). The human RANK L cDNA encodes 317 amino acids (aa), including a 47 aa cytoplasmic domain, a 21 aa TM region, and a 249 aa extracellular domain (ECD) with two potential N-linked glycosylation sites (note: Arg85-Asp245 of Accession # AAC51762 is identical to Arg157-Asp317 of SwissProt # O14788. This aa range contains the ECD trimerization and receptor-binding motifs, but not ECD proteolytic cleavage sites). Within the ECD, human RANK L shares 89%, 89%, 93% and 95% aa identity with mouse, rat, bovine and porcine RANK L, respectively. Mouse RANK L can stimulate human osteoclast differentiation (4). Like most TNF family members, RANK L can form trimers (1). Soluble 31, 25 and 24 kDa forms of RANK L can be created by usage of alternate start sites at aa 74 or 146, or proteolytic cleavage by osteoblast- or stromal cell-derived ADAM10 (after aa 139) or MMP14 (aa 146), or bone metastatic prostate tumor-derived MT1-MMP (aa 146) (5, 7, 8). Both TM and soluble extracellular RANK L act by engaging RANK receptors and are antagonized by the decoy receptor, OPG (osteoprotegerin) (2, 5). In resting cells, the majority of RANK L is stored in secretory lysosomes (9). In mammary epithelia, RANK L is upregulated by pregnancy hormones and is essential for the formation of a lactating mammary gland (10). In the brain, astrocyte RANK L mediates body temperature regulation (11). Pathologically, RANK L is thought to mediate post-menopausal osteoporosis, vascular calcification, progestin-induced breast cancer, cancer-induced bone disease, and osteopetrosis (in RANK L deficiencies) (12-16). Our Avi-tag Biotinylated human TRANCE/RANK L features biotinylation at a single site contained within the Avi-tag, a unique 15 amino acid peptide. Protein orientation will be uniform when bound to streptavidin-coated surface due to the precise control of biotinylation and the rest of the protein is unchanged so there is no interference in the protein's bioactivity.

References:

1. Leibbrandt, A. and J.M. Penninger (2008) *Ann. N.Y. Acad. Sci.* **1143**:123.
2. Wong, B.R. *et al.* (1997) *J. Biol. Chem.* **272**:25190.
3. Anderson, D.M. *et al.* (1997) *Nature* **390**:175.
4. Lacey, D.L. *et al.* (1998) *Cell* **93**:165.
5. Hikita, A. *et al.* (2006) *J. Biol. Chem.* **281**:36846.
6. Kong, Y.-Y. *et al.* (1999) *Nature* **397**:315.
7. Accession # NP_143026 and EAX08679.
8. Sabbota, A.L. *et al.* (2010) *Cancer Res.* **70**:5558.
9. Aoki, S. *et al.* (2010) *J. Bone Miner. Res.* **25**:1907.
10. Fata, J.E. *et al.* (2000) *Cell* **103**:41.
11. Hanada, R. *et al.* (2009) *Nature* **426**:505.
12. Osako, M.K. *et al.* (2010) *Circ. Res.* **107**:466.
13. Schramek, D. *et al.* (2010) *Nature* **468**:98.
14. Gonzalez-Suarez, E. *et al.* (2010) *Nature* **468**:103.
15. Dougall, W.C. and M. Chaisson (2006) *Cancer Metastasis Rev.* **25**:541.
16. Sobacchi, C. *et al.* (2007) *Nat. Genet.* **39**:960.