

## Product Information

### RANK Ligand/TRANCE human

recombinant, expressed in NSO cells

Product Number **T3573**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

Synonyms: Osteoclast Differentiation Factor (ODF), Receptor Activator of NF- $\kappa$ B Ligand (RANKL), TNF-related activation-induced cytokines (TRANCE), Osteoprotegerin Ligand (OPGL)

#### Product Description

RANK Ligand (receptor activator of NF- $\kappa$ B ligand (RANKL), also known as TNF-related activation-induced cytokines (TRANCE), osteoprotegerin ligand (OPGL), and osteoclast differentiation factor (ODF), is a member of the tumor necrosis factor (TNF) family. It was originally identified as an immediate early gene upregulated by T cell receptor stimulation. RANK Ligand/TRANCE is a type II transmembrane protein of 317 amino acids with a predicted cytoplasmic domain of 47 amino acids, a 21 amino acid transmembrane region, and an extracellular domain of 249 amino acids. The extracellular domain contains two potential N-linked glycosylation sites. Mouse and human RANK Ligand/TRANCE share 85% amino acid identity.

RANK Ligand/TRANCE is a key regulator of the immune system, and of bone development and homeostasis.<sup>1</sup> The multi-functions of RANK Ligand/TRANCE include induction of activation of the c-jun N-terminal kinase,<sup>2</sup> enhancement of T cell growth and dendritic cell function,<sup>3</sup> induction of osteoclastogenesis,<sup>3,4</sup> and lymph node organogenesis.<sup>3</sup> The cell surface signaling receptor of RANK Ligand/TRANCE is RANK, which undergoes receptor clustering during signal transduction.

RANK Ligand/TRANCE is primarily expressed in T cells and T cell rich organs, such as thymus and lymph nodes. It is abundantly expressed in T cells but not in B cells.<sup>2</sup> RANK Ligand/TRANCE activates mature dendritic cells, inducing cytokine production, suggesting it is a factor in the T cell-dendritic cell interaction during an immune response.<sup>5</sup> Osteoblasts, expressing RANK Ligand/TRANCE, regulate osteoclast differentiation.<sup>6</sup>

Recombinant, human RANK Ligand/TRANCE is expressed with amino acid residues (Gly<sup>136</sup>-Asp<sup>317</sup>) of the extracellular domain of human TRANCE/RANK Ligand<sup>7</sup> fused to the signal peptide of CD33 and a polypeptide linker. It is expressed in mouse myeloma NSO cells.

The recombinant, human RANK Ligand/ TRANCE product, generated after removal of the 16 amino acid residue signal peptide, contains 209 amino acids with a calculated mass of 23 kDa. Due to glycosylation, the recombinant protein migrates to  $\sim 35$  kDa in SDS-PAGE under reducing and non-reducing conditions.

The recombinant product is supplied as  $\sim 10\text{ }\mu\text{g}$  of protein lyophilized from a  $0.2\text{ }\mu\text{m}$  filtered solution in 20 mM MOPS and 500 mM NaCl, pH 6.5, with  $50\text{ }\mu\text{g}$  BSA per  $1\text{ }\mu\text{g}$  as a carrier protein.

Purity:  $>90\%$  (SDS-PAGE)

The biological activity of Rank Ligand/TRANCE is measured by its ability to induce osteoclast differentiation on mouse splenocytes. The  $\text{ED}_{50}$  for this effect is 1.5–7.5 ng/ml in the presence of  $2.5\text{ }\mu\text{g/ml}$  of a crosslinking mouse Anti-6X-histidine antibody.

Endotoxin:  $<0.1\text{ ng}/\mu\text{g}$  of protein  
[Limulus amoebocyte lysate (LAL) method]

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

Reconstitute the contents of the vial using  $0.2\text{ }\mu\text{m}$  filtered phosphate buffered saline containing at least 0.1% human serum albumin or bovine serum albumin. Prepare a stock solution of no less than  $10\text{ }\mu\text{g/ml}$ .

### Storage/Stability

Store the product at –20 °C.

The reconstituted product may be stored at 2–8 °C for up to one month. For extended storage, freeze working in aliquots. Repeated freezing and thawing is not recommended. Do not store in a “frost-free” freezer.

### References

1. Wong, B.R., et al., TRANCE is a TNF family member that regulates dendritic cell and osteoclast function. *J. Leukoc. Biol.*, **65**, 725-724 (1999).
2. Wong, B.R., et. al., TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. *J. Biol. Chem.*, **272**, 25190-25194 (1997).
3. Kong, Y.Y., et. al., OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*, **397**, 315-323, (1999).
4. Nakagawa, N., et. al., RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis, *Biochem. Biophys. Res. Commun.*, **253**, 395-400 (1998).
5. Josien, R., et al., TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. *J. Immunol.*, **162**, 2562-2568 (1999).
6. Takahashi, N. et al., A new member of tumor necrosis factor ligand family, ODF/OPGL/ TRANCE/RANKL, regulates osteoclast differentiation and function. *Biochem. Biophys. Res. Commun.*, **256**, 449-455 (1999).
7. Anderson, D.M., et. al., A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature*, **390**, 175-179 (1997).

SG,ANK,PHC,MAM,AI 02/17-1