



Product Information Sheet

Human Receptor activator of NF κ B, RANK ELISA Kit

Catalog No. EK0829

Size 96T

Range 62.5pg/ml-4000pg/ml

Sensitivity < 2 pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Storage

Store at 4 °C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

Four months at 4°C and eight months at -20°C.

Application

For quantitative detection of human RANK in tissue lysates or cell culture supernates, and should be confirmed for serum and plasma.

Principle

Human RANK ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human RANK specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human RANK amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human RANK standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human RANK antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human RANK antibody: 130 μ l, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130 μ l, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended if there is a large amount of samples.
3. Clean tubes and Eppendorf tubes.
4. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450 μ l of purified acetic acid or 700 μ l of concentrated hydrochloric acid to 1000ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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Antagene, Inc.

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Tel: (650) 964-2589

Fax: (650) 964-2519

email: Info@antageneinc.com

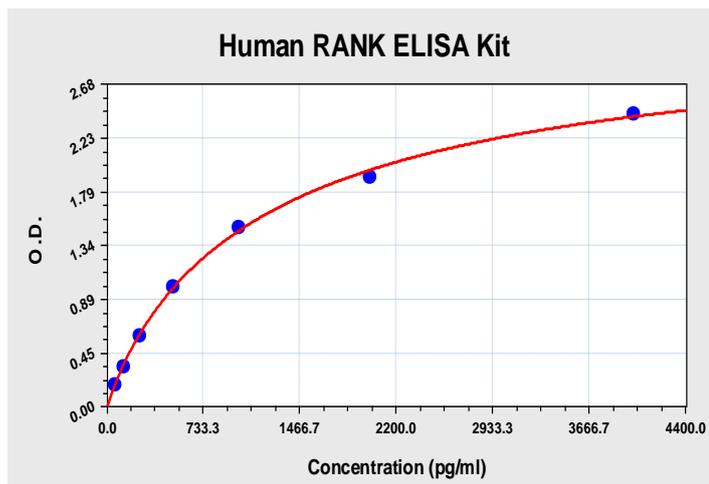
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Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Human RANK ELISA Kit-1X96 Well Plate Image



Background

Receptor Activator of Nuclear Factor κ B (RANK), also known as TRANCE Receptor, is a type I membrane protein that is expressed on the surface of osteoclasts and is involved in their activation upon ligand binding. RANK is a recently described TNF receptor family member, and its ligand, RANKL, promote survival of dendritic cells and differentiation of osteoclasts. RANK contains 383 amino acids in its intracellular domain (residues 234-616), which contain three putative TRAF-binding domains (termed I, II, and III). Darnay BG et al demonstrated that RANK interacts with various TRAFs through distinct motifs and activates NF-kappaB via a novel TRAF6 interaction motif, which then activates NIK, thus leading to NF-kappaB activation, whereas RANK most likely activates JNK through a TRAF2-interacting region in RANK. The standard in this kit is recombinant human RANK with the sequence of Q29-G213 aa. It is a dipolymer which compose of two chains, and the molecular weight of each is 48kda.

Reference

1. Darnay, B G; Haridas V, Ni J, Moore P A, Aggarwal B B (Aug. 1998). "Characterization of the intracellular domain of receptor activator of NF-kappaB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-kappab and c-Jun N-terminal kinase". *J. Biol. Chem.* (UNITED STATES) **273** (32): 20551–5.
2. Darnay, B G; Ni J, Moore P A, Aggarwal B B (Mar. 1999). "Activation of NF-kappaB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-kappaB-inducing kinase. Identification of a novel TRAF6 interaction motif". *J. Biol. Chem.* (UNITED STATES) **274** (12): 7724–31.

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