Human RANK ELISA kit

Catalog No.: abx050195

Size: 96T

Range: 62.5 pg/ml - 4000 pg/ml

Sensitivity: < 2 pg/ml

Storage: Store at 2-8°C for 4 months, or at -20°C for 8 months.

Application: For quantitative detection of RANK in Human Serum, Cell Culture Supernatants and plasma.

Introduction: Receptor Activator of Nuclear Factor κ B (RANK), also known as TRANCE Receptor or TNFRSF11A, is a member of the tumor necrosis factor receptor (TNFR) molecular sub-family. RANK is the receptor for RANK-Ligand (RANKL) and part of the RANK/RANKL/OPG signaling pathway that regulates osteoclast differentiation and activation. It is associated with bone remodeling and repair, immune cell function, lymph node development, thermal regulation, and mammary gland development. Osteoprotegerin (OPG) is a decoy receptor for RANK, and regulates the stimulation of the RANK signaling pathway by competing for RANKL. The cytoplasmic domain of RANK binds TRAFs 1, 2, 3, 5, and 6 which transmit signals to downstream targets such as NF-κB and JNK. RANK is constitutively expressed in skeletal muscle, thymus, liver, colon, small intestine, adrenal gland, osteoclast, mammary gland epithelial cells, prostate, vascular cell, and pancreas. Most commonly, activation of NF-κB is mediated by RANKL, but over-expression of RANK alone is sufficient to activate the NF-κB pathway. RANKL (Receptor Activator for Nuclear factor κ B Ligand) is found on the surface of stromal cells, osteoblasts, and T cells.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-RANK antibody is pre-coated onto 96-well plates. Biotin conjugated antibody is used as a detection antibody. The standards, test samples and biotin conjugated antibody are added to the wells and washed with wash buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the SDF-1 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of RANK can be calculated.

Kit components

1. One 96 well plate.
2. Standard: 2 tubes
3. Sample / Standard diluent buffer: 30ml
4. Biotin conjugated antibody: 130µl - Dilution: 1:100
5. Antibody diluent buffer: 12 ml
6. Avidin-Biotin-Peroxidase Complex (ABC): 130 µl - Dilution: 1:100
7. ABC diluent buffer: 12 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma with EDTA as the anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. **Heparin or citrate cannot be used as anticoagulant.**

Note:

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» NaN3 cannot be used as test sample preservative, since it inhibits HRP.

General Sample guideline:

Estimate the concentration of the target protein in the sample and select the correct dilution factor to make the diluted target protein concentration fall near the middle of the kit's range. For high concentration, dilute 1:100, for medium concentration, dilute 1:10 and for low concentration, dilute 1:2. Very low concentrations do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

The standard solution should be prepared no more than 2 hours prior to the experiment. Note: Do not dilute the standard directly in the plate. Two tubes of standard are included in each kit. Use one tube for each experiment.

a.) Preparing the standard: Add 1 ml of Sample/Standard diluent buffer into one Standard tube to make the 10000 pg/ml solution. Keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.

b.) 4000 pg/ml of standard solution: Add 0.4 ml of the above 10 ng/ml standard solution into 0.6 ml sample diluent buffer and mix thoroughly.

b.) 2000 pg/ml → 62.5 pg/ml standard solutions: Label 7 tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml and 62.5 pg/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 4000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from the 1st tube to the 2nd tube and mix thoroughly. Transfer 0.3 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.

10000 pg/ml
RANK Solution

0.3 ml 0.3 ml 0.3 ml 0.3 ml 0.3 ml 0.3 ml

4000 2000 1000 500 250 125 62.5
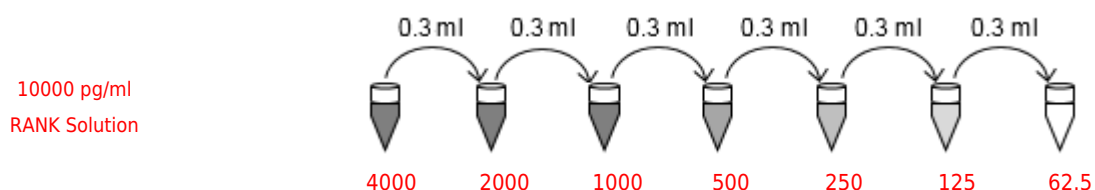
Note: The standard solutions are best used within 2 hours. The standard solution should be used within 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 2 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the ABC with ABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of ABC into 99 µl of ABC diluent buffer.

B. Assay Procedure

Before adding to wells, equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (18-25℃). It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommend to measure each standard and sample in duplicate or triplicate.

2. Aliquot 0.1ml of the diluted standards into the standard wells.

3. Add 0.1 ml of Sample / Standard diluent buffer into the control (zero) well.

4. Add 0.1 ml of appropriately diluted sample into test sample wells.

5. Seal the plate with a cover and incubate at 37℃ for 90 min.

6. Remove the cover and discard the plate content, tap the plate on absorbent filter papers. **Do not let the wells completely dry at any time. Do not wash plate.**

7. Add 0.1 ml of Biotin conjugated antibody working solution into the wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.

8. Seal the plate with a cover and incubate at 37℃ for 60 min.

9. Remove the cover, and wash plate 3 times with Wash buffer using one of the following methods:

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent filter papers. Fill each well completely with Wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers.

Automated Washing: Discard the solution and wash the plate three times with Wash buffer (overfilling the wells with the buffer). After the final wash, tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37℃ for 30 min.

11. Remove the cover and wash the plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.(See Step 9 for plate wash method).

12. Add 0.1 ml of TMB substrate into each well, cover the plate and incubate at 37℃ in dark conditions for 15-30 min. (Note: This incubation time is for reference only, the optimal time should be determined by the end user.)

13. Add 0.1 ml of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

14. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450nm immediately.

For calculation: (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human RANK concentration of the samples can be interpolated from the standard curve.
Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.Equilibrate the ABC working solution for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

C. Precautions

1. Before the experiment, centrifuge the tubes briefly to bring down any contents trapped in the lid.

2. Avoid foaming or bubbles when mixing or reconstituting components.

3. It is recommend to assay all standards, controls and samples in duplicate or triplicate.

4. Do NOT let the plate completely dry at any time as this will inactivate the biological material on the plate

5. Ensure plates are properly sealed or covered during incubation steps.

6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

7. Do not reuse pipette tips and tubes to avoid cross contamination.

8. Do not use expired components or components from different batches.

9. Store the TMB substrate in the dark and to avoid the edge effect of plate incubation for temperature differences it is recommend to equilibrate the ABC working solution and TMB substrate for at least 30 min at room temperature (18-25℃) before adding to wells.

10. The TMB substrate is colorless and transparent before use, if not, please contact us for replacement.

D. Typical Data & Standard Curve
Results of a typical standard run of a Human RANK ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

pg/ml	0	62.5	125	250	500	1000	2000	4000
OD450	0.052	0.088	0.119	0.213	0.401	0.659	1.281	2.284