Mouse sRANK Ligand / TNFSF11 / TRANCE ELISA Kit

Enzyme Immunoassay for the quantification of Mouse soluble Receptor Activator of NFkB Ligand in serum, plasma, cell culture supernatants

Catalog number: ARG80222

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene encodes a member of the tumor necrosis factor (TNF) cytokine family which is a ligand for osteoprotegerin and functions as a key factor for osteoclast differentiation and activation. This protein was shown to be a dentritic cell survival factor and is involved in the regulation of T cell-dependent immune response. T cell activation was reported to induce expression of this gene and lead to an increase of osteoclastogenesis and bone loss. This protein was shown to activate antiapoptotic kinase AKT/PKB through a signaling complex involving SRC kinase and tumor necrosis factor receptor-associated factor (TRAF) 6, which indicated this protein may have a role in the regulation of cell apoptosis. Targeted disruption of the related gene in mice led to severe osteopetrosis and a lack of osteoclasts. The deficient mice exhibited defects in early differentiation of T and B lymphocytes, and failed to form lobulo-alveolar mammary structures during pregnancy. Two alternatively spliced transcript variants have been found. [provided by RefSeq, Jul 2008]
Cytokine that binds to TNFRSF11B/OPG and to TNFRSF11A/RANK. Osteoclast differentiation and activation factor. Augments the ability of dendritic cells to stimulate naive T-cell proliferation. May be an important regulator of interactions between T-cells and dendritic cells and may play a role in the regulation of the T-cell-dependent immune response. May also play an important role in enhanced bone-resorption in humoral hypercalcemia of malignancy (By similarity). Induces osteoclastogenesis by activating multiple signaling pathways in osteoclast precursor cells, chief among which is induction of long lasting oscillations in the intracellular concentration of Ca (2+) resulting in the activation of NFATC1, which translocates to the nucleus and induces osteoclast-specific gene transcription to allow differentiation of osteoclasts (PubMed:24039232). During osteoclast differentiation, in a TMEM64 and

ATP2A2-dependent manner induces activation of CREB1 and mitochondrial ROS generation necessary for proper osteoclast generation (PubMed:23395171, PubMed:26644563). [provided by uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for sRANK Ligand / TNFSF11 / TRANCE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any sRANK Ligand / TNFSF11 / TRANCE present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for sRANK Ligand / TNFSF11 / TRANCE is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of sRANK Ligand / TNFSF11 / TRANCE bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of sRANK Ligand / TNFSF11 / TRANCE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	3 X 2 ng/vial	4°C
Standard diluent buffer	20 ml	4°C
Antibody conjugate concentrate	1 vial (400 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	1 vial (400 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	6 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

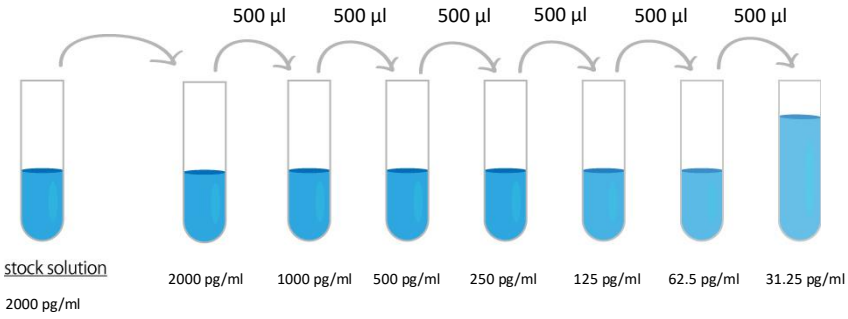
Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Antibody conjugate: 20 minutes before use, dilute 30X antibody conjugate concentrate into 1X antibody diluent buffer to yield 1X Detection antibody solution.
- 1X HRP-Streptavidin Solution: 20 minutes before use, dilute 30X HRP-Streptavidin concentrate solution into 1X HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- Standards: Reconstitute the standard with 1 ml standard diluent buffer to yield a stock concentration of 2000 pg/ml. Make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml.



- Sample: If the initial assay found samples contain sRANK Ligand / TNFSF11 / TRANCE higher than the highest standard, the samples can be diluted with Standard diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
  (It is recommended to do pre-test to determine the suitable dilution factor).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (standard diluent buffer) into wells. Incubate for 1.5 h at 37°C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 37°C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and

incubate for 30 minutes at 37°C.

7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
10. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

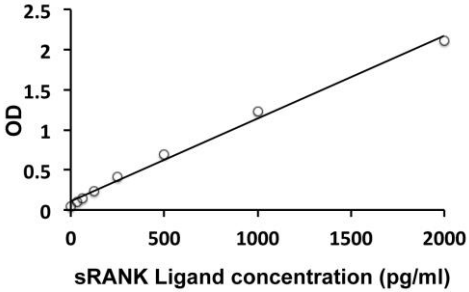
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Mouse sRANK Ligand / TNFSF11 / TRANCE ranged from 31.25-2000 pg/ml. The mean MDD was 15 pg/ml.

Specificity

This assay recognizes natural and recombinant Mouse sRANK Ligand / TNFSF11 / TRANCE. No significant cross-reactivity or interference with the factors below was observed:

Mouse C10 MIP-1, JE MIP-1 , KC; human GROβ, GROγ, RANTES

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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