Version 17-04/24

User's Manual

FREE soluble RANKL High Sensitivity ELISA kit

REF **DEIA22314**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

3rd Generation ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF FREE, SOLUBLE, UNCOMPLEXED HUMAN RANKL IN SERUM OR HEPARIN PLASMA.

General Description

RANKL, the receptor activator of nuclear factor kappa B ligand, a member of the tumor necrosis factor (TNF) family, is the main stimulatory factor for the formation ofmature osteoclasts and is essential for their survival. RANKL activates its specific receptor RANK, located on osteoclasts and dendritic cells. The effects are counteracted by OPG which acts as an endogenous soluble receptor antagonist.

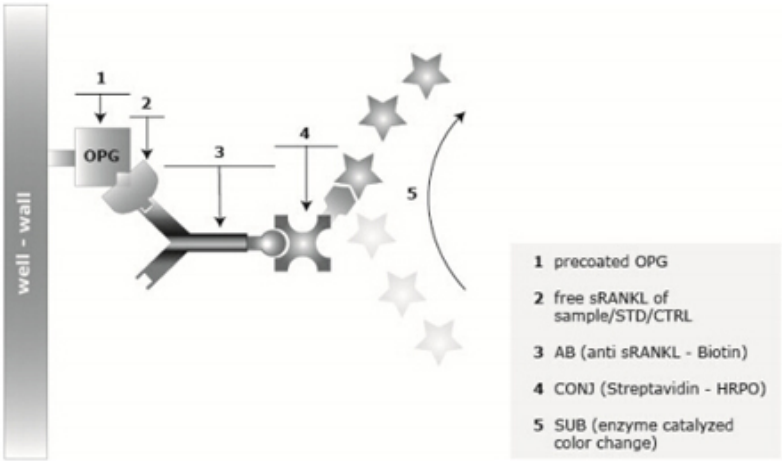
The major source of RANKL are osteocytes, former osteoblasts that become embedded within the mineralized bone matrix. RANKL is a ~35 kD type II transmembrane-type protein and is cleaved to release a soluble biologically active product that forms a homotrimer.

RANKL and its specific receptor RANK are not only key regulators of bone remodeling but also play an essential role in immunobiology, e.g. lymph node formation, establishment of the thymic microenviroment, mammarygland development during pregnancy, bone metastasis in cancer and sex-hormone, progestin-driven breast cancer, thermoregulation, and finally in the development of type 2 diabetes mellitus.

Possible Indications:

1) Postmenopausal and senile osteoporosis
2) Glucocorticoid induced osteoporosis
3) Disease with locally increased resorption activity
4) Arthritis
5) Oncology
6) Type 2 diabetes mellitus

Principles of Testing



Reagents And Materials Provided

CONT	KIT COMPONENTS	QUANTITY
PLATE	Human recombinant OPG pre-coated microtiter strips in strip holder	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 7 ml
AB	Goat polyclonal biotinylated anti sRANKL antibody, green cap, ready to use	1 x 22 ml
STD	Standards (0; 0.0625; 0.125; 0.25; 0.5; 1; 2 pmol/l), white caps	7 vials lyophilised
CTRL	Controls, recombinant human RANKL in human serum, yellow caps, exact concentration after reconstitution see label	2 vials lyophilised
CONJ	Conjugate (streptavidin-polyHRPO), amber cap, ready to use	1 x 22 ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready to use	1 x 22 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

ADDITIONAL MATERIAL SUPPLIED IN THE KIT

1) 3 self-adhesive plastic films
2) QC protocol
3) Protocol sheet
4) Instruction manual for use

Materials Required But Not Supplied

1. Precision pipettes calibrated to deliver 50 µL, 150µL, 200 µL, 300 µL and disposable tips
2. Distilled or deionised water
3. Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
4. Refrigerator with 4°C (2-8°C)
5. ELISA reader capable of measuring absorbance at 450nm (with correction wavelength at 630 nm)
6. Graph paper or software for calculation of results

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Specimen Collection And Preparation

All reagents of the kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or Heparin plasma.We recommend performing plasma or serum separation by centrifugation as soon as possible, e.g. 10 min at 2000 x g, preferably at 4°C (2-8°C). If this is not possible, store the samples at 4°C (2-8°C) prior to centrifugation (maximal one day).The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Samples should undergo 3 freeze-thaw cycles only. Lipemicor haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values. If samples read higher than the top standard, we recommend diluting with a lowmeasuring serum sample and re-measuring the samples.

Reconstitution And Storage

STD (Standards) and CTRL (Controls): Pipette 700 µL of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 15 min. Reconstituted STD and CTRL are stable at -25°C or lower until expiry date stated on label. STDs and CTRLs are stable for3 freeze-thaw cycles.

Reagent Preparation

WASHBUF (Wash buffer): Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature. The undiluted buffer is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stableup to one month at 4°C (2-8°C). Only use diluted WASHBUF (Wash buffer) when performing the assay.

Assay Procedure

All reagents and samples must be at room temperature (18-26°C) before use in the assay.

Mark position for BLANK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.

Take microtiter strips out of the aluminium bag, take a minimum of one well as blank. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1. **Prewash wells** with 300 µL diluted WASHBUF (wash buffer, natural cap) five times. Remove remaining WASHBUF by tapping plate against paper towel after the last wash.
2. Add 50 µL ASYBUF (assay buffer, red cap) into each well. Pipette additional 150 µL ASYBUF into well marked as blank.
3. Pipette 150 µL STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells, except blank.

4. Cover tightly and incubate at room temperature (18-26°C) for 2 hours.

5. Aspirate and wash wells with 300 µL diluted WASHBUF(wash buffer, natural cap) five times. Remove remaining WASHBUF by tapping plate against paper towel after the last wash.
6. Add 200 µL AB (biotinylated anti sRANKL antibody, green cap) into each well, except blank. Swirl gently. Pipette additional 200 µL ASYBUF (assay buffer, redcap) into well marked as blank.

7. Cover tightly and incubate at 4°C (2-8°C) over night (18-24 hours).

8. Aspirate and wash wells with 300 µL diluted WASHBUF(wash buffer, natural cap) fives times. Remove remaining WASHBUF by tapping plate against paper towel after the last wash.
9. Add 200 µL CONJ (conjugate, amber cap) into each well.

10. Cover tightly and incubate at room temperature (18-26°C) for 1 hour in the dark.

11. Aspirate and wash wells with 300 µL diluted WASHBUF(wash buffer, natural cap) fives times. Remove remaining WASHBUF by tapping plate against paper towel after the last wash.
12. Add 200 µL SUB (substrate, blue cap) into each well.

13. Incubate at room temperature (18-26°C) for 30 min in the dark.

14. Add 50 µL STOP (stop solution, white cap) into eachwell.

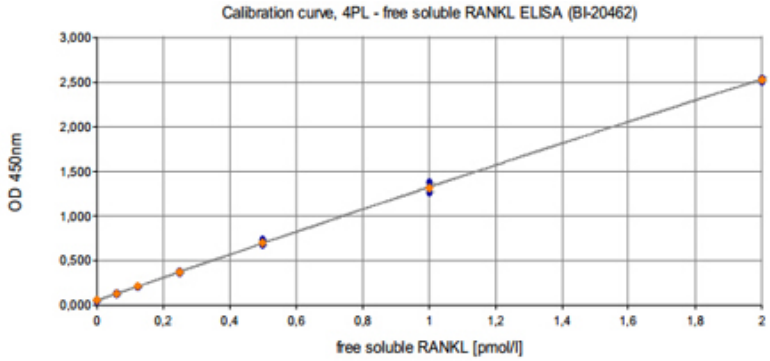
15. Measure absorbance immediately at 450 nm with reference 630 nm if available.

Calculation

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Subtract the blank OD from the values of STD, CTRL and sample. Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Calculate sample concentration from the standard curve. The assay was evaluated with 4PL algorithm. Different curve-fitting methods need to be evaluated by the user.

Typical Standard Curve

Example typical STD-curve:



The quality control protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for optical density obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.50 or more is obtained for the standard with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

Performance Characteristics

Values of apparently healthy individuals:	Median (serum, n = 32): 0.14 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during study.
Standard range:	0; 0.0625; 0.125; 0.25; 0.5; 1; 2 pmol/l
Conversion factor pg/ml to pmol/l:	1 pg/ml = 0.05 pmol/l (MW: 20 kD, monomer)
Sample volume:	150 µl human serum or Heparin plasma
Detection limit / LLOQ:	(0 pmol/l + 3 SD): 0.01 pmol/l / 0.008pmol/l
Incubation time:	2 h / overnight / 1 h / 30 min

Precision

Intra-assay: 2 samples of known concentrations weretested 5 times within one kit lot by one operator.

Inter-assay: 2 samples of known concentrations weretested 12 times within 3 different kit lots and by3 different operators.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=12)	Sample 1	Sample 2
Mean (pmol/l)	0.12	1.00	Mean (pmol/l)	0.12	1.00
SD (pmol/l)	0.005	0.04	SD (pmol/l)	0.004	0.02
CV (%)	4	4	CV (%)	3	2

Precautions

TECHNICAL HINTS

1. Do not mix or substitute reagents with those from other lots or sources.
2. Do not mix stoppers and caps from different reagents or use reagents between lots.
3. Do not use reagents beyond expiration date.
4. Protect reagents from direct sunlight.
5. Substrate solution should remain colourless until added to the plate.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
7. Avoid foaming when mixing reagents.

PRECAUTIONS

All test components of human source were tested against HIV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.

All liquid reagents contain ≤0.1% Proclin 300 as preservative. Avoid contact with skin and mucous membrane. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions - avoid contact with skin or eyes.

1. Do not pipette by mouth.
2. Do not eat, drink, smoke or apply cosmetics where reagents are used.
3. Wear gloves, glasses and lab coat while performing this assay.
4. Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs!!

Limitations

Serum/plasmasamples with totals RANKL levels greater than the highest standard value, should be diluted with wash buffer and re-assayed.