Manual

sRANKL ELISA Kit

For the in vitro determination of mouse/rat sRANKL in serum and cell culture supernatant

For research use only

Valid from 24.05.2011

REF **K 1019**

Σ 96

+ 8 °C
+ 2 °C

RUO

Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim
Tel.: ++49 6251 70190-0
Fax: ++ 49 6251 849430
e.mail: Info@immundiagnostik.com
www.Immundiagnostik.com

Inhaltsverzeichnis 1

Table of contents 2

1. INTENDED USE

The Immundiagnostik assay is a sandwich ELISA intended for the quantitative determination of mouse/rat sRANKL in serum and cell culture supernatant. It is for research use only.

2. SUMMARY AND EXPLANATION OF THE TEST

RANKL, receptor activator of nuclear factor (NF)-κB ligand (also: osteoprotegerin ligand, OPGL), its cellular receptor, receptor activator of NF-κB (RANK), and the decoy receptor, osteoprotegerin (OPG) have been identified as the key molecular regulation system for bone remodelling. RANKL, a member of the tumor necrosis factor (TNF) family, is the main stimulatory factor for the formation of mature osteoclasts and is essential for their survival. Therefore, an increase in RANKL expression leads to bone resorption and bone loss. RANKL is produced by osteoblastic lineage cells and activated T lymphocytes. It activates its specific receptor RANK which is located on osteoclasts and dendritic cells.

The effects of RANKL are counteracted by OPG which is secreted by various tissues and acts as an endogenous soluble receptor antagonist.

Imbalances of the RANKL/OPG system have been related to the pathogenesis of Paget's disease, benign and malignant bone tumors, postmenopausal osteoporosis, rheumatoid arthritis, bone metastases and hypercalcemia. It was shown in several studies that in animal models restoring of the RANKL/OPG balance (e.g. by administering OPG) reduces the severity of these disorders.

It has been shown that RANKL is produced as a membrane-bound protein on murine osteoblasts/stromal cells, and cleaved into a soluble form by a metalloprotease. Stimulators of the osteoclastogenesis such as IL-1beta, IL-6, IL-11, IL-17, and TNF-alpha, increase the expression of RANKL and decrease OPG expression in osteoblasts/stromal cells. Cytokines inhibiting the osteoclastogenesis such as IL-13, INF-gamma, and TGF-beta1, suppress the expression of RANKL and stimulated OPG expression.

Indications

- Postmenopausal and senile osteoporosis
- Diseases with locally increased bone resorption activity
- Paget´s disease
- Periodontal disease
- Inflammatory diseases
- Immunological disorders
- Arthritis
- Oncology

3. PRINCIPLE OF THE TEST

This sandwich-type ELISA is an assay for the direct determination of sRANKL in serum, plasma and urine. In this assay two highly specific antibodies against sRANKL are used. The capture antibody is attached to the wells of the microtiter plate, the detection antibody is labeled with biotin.

In a first incubation step the samples and the biotinylated antibody against sRANKL react with the coated capture antibody on the microtiter plate. A sandwich-type complex is formed consisting of the binding antibody on the plate, sRANKL and the biotinylated detection antibody. To remove all unspecific bound substances a washing step is carried out.

In a second step streptavidin – peroxidase is added which reacts with the detection antibody. After another washing step, the solid phase is incubated with the substrate, TMB. An acidic stopping solution is subsequently added. The blue color changes to yellow. The intensity of the yellow color is directly proportional to the concentration of sRANKL in the sample.

A dose - response curve of the absorbance units at 450 nm versus concentration is generated. sRANKL in the samples is determined directly from this calibration curve.

4. MATERIAL SUPPLIED

Cat. no.	Label	Kit Components	Quantity
K1019LMTP	PLATE	Microtiter plate, 12 x 8 strips	96
K1019WP	WASHBUF	ELISA washing buffer concentrate (10x)	100 ml
K1019BeP	COATBUF	Coating buffer	30 ml
K1019A	COATAB	Capture antibody (rat anti-mouse RANKL), lyophilized	1 vial
K1019A2	2.AB	Detection antibody (goat anti-mouse RANKL, biotinylated), lyophilized	1 vial
K1019ST	STD	Calibrator (4000 pg) lyophilized	2 vials
K1019K	CONJ	Conjugate, (Strepdavidin-HRP-labeled), ready-to-use	1 vial
K1019DL	DIL	Reagent Diluent	100 ml
K1019TMB	SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	22 ml
K1019AC	STOP	ELISA stop solution, ready-to-use	1 x 15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Precision pipettes calibrated to deliver 50 -1000 µl and disposable tips.
- Multichannel or Multipipette
- Vortex mixer
- Conventional glass or plastic tubes
- 1,5 ml reaction vials (Eppendorf)
- ELISA reader equipped with 450 nm filter

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- The **WASHBUF** (ELISA wash buffer concentrate) should be diluted with aqua bidist. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidist.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C before dilution. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- **COATAB** (Capture Antibody, 1 vial), 144 µg/ml of rat anti-mouse RANKL Reconstitute with **35 µl bidistilled water**. After reconstitution, store at 2 - 8°C for up to 60 days or aliquot and store at -20°C to -70°C for up to 6 months. Dilute immediately before use to a **working concentration of 0.4 µg/ml in COATBUF** (coating buffer).

- **2.AB (Detection Antibody, 1 vial),** 36 µg/ml of biotinylated goat anti-mouse RANKL. Reconstitute with **70 µl bidistilled water**. After reconstitution, store at 2 – 8°C for up to 60 days or aliquot and store at -20°C to -70°C for up to 6 months. Dilute to a **working concentration of 200 ng/ml in DIL** (Reagent Diluent).

- **STD (Standard, 1 vial),** 4000 pg/ml of recombinant mouse RANKL Reconstitute with **0.6 mL bidistilled water**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Store reconstituted standard at 2 - 8° C max. overnight or aliquot and store at -70° C for up to 2 months. A seven point standard curve using **2-fold serial dilutions in DIL** (Reagent Diluent), and a high standard of 4000 pg/ml is recommended.
- **CONJ (Streptavidin-HRP, 1 vial),** 60 µl of streptavidin conjugated to horseradish-peroxidase. Store at 2 - 8° C. **Do not freeze**. Dilute to the working concentration **1:200 in DIL** (Reagent Diluent).

7. PRECAUTIONS

- To avoid cross-contamination, change pipette tips between addition of standards, controls, samples, antibody, conjugate and substrate. Also use separate reservoirs for each reagent.
- Avoid foaming when mixing the reagents.
- Do not mix stoppers and caps of different reagents.
- Do not use reagents beyond expiration date.
- Do not mix or substitute reagents with those from other lots or sources.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate solution should remain colourless until added to the plate.
- Stop solution should be added to the plate in the same order as the substrate solution.
- Protect reagents from direct sunlight.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

8. SPECIMEN COLLECTION AND PREPARATION

Serum, plasma and urine samples

Serum, plasma and urine samples can be used without any dilution. Serum must be centrifuged and aliquoted within 90 min after collection and stored at –20 °C until use.

9. ASSAY PROCEDURE

Procedural notes

- Do not mix different lot numbers of any kit component.
- Guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.

Plate Preparation

1. Coat a 96-well microplate with **100 µL per well of the diluted COATAB** (capture antibody). Seal the plate and incubate overnight at room temperature.

2. Aspirate and wash the wells **5 x with 250 µl** ELISA wash buffer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Block plates by adding **250 µl of DIL** (reagent diluent) to each well. Incubate at room temperature for 1 hour.

4. Aspirate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the last wash, remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels. The plates are now ready for sample addition.

Assay Procedure

1. Add **100 µl of SAMPLE** (sample) or **STD** (standards) in Reagent Diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.

2. Aspirate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the last wash, remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Add **100 µl of the 2. AB** (detection antibody), diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.

4. Aspirate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the last wash, remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

5. Add **100 µl of the working dilution of CONJ** (streptavidin-HRP) to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

6. Aspirate and wash the wells **5 x with 250 µl** ELISA wash buffer. After the last wash, remove any remaining wash buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

7. Add **100 µl of SUB** (substrate solution) to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.

8. Add **50 µl of STOP** (stop solution) to each well. Gently tap the plate to ensure thorough mixing.

9. Determine absorption immediately with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

10. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

2. Point-to-point-calculation

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

11. LIMITATIONS

Samples with sRANKL levels greater then the highest standard value should be further diluted and re-assayed.

12. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

13. REFERENCES

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14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The test components which are made of human serum are tested for Australia antigen and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- For research use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not mix different lot numbers of any kit component.
- Quality control guidelines should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

Used symbols:


Temperature limitation

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device


Contains sufficient for <n> tests


Manufacturer


Use by

LOT Lot number

RUO For research use only