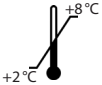
Manual

Osteoprotegerin ELISA

For the in vitro determination of of mouse/rat OPG in serum, plasma, urine and cell culture supernatant

Valid from 2020-09-17

REF **KR1020**



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1. INTENDED USE

This Immundiagnostik AG assay is a sandwich ELISA intended for the determination of mouse/rat Osteoprotegerin in serum, plasma, urine and cell culture supernatants.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

Osteoprotegerin (OPG) or Osteoclastogenesis inhibitory factor (OCIF) is a dimeric glycoprotein of the TNF receptor family with a molecular weight of 60 kD resp. 120 kD which shows an inhibitory effect on osteoclasts and osteoclast precursor cells.

Osteoprotegerin is a soluble decoy receptor and is produced in different tissues, e. g. bone, skin, liver, stomach, intestine and lung. As a so-called decoy receptor OPG inhibits the binding of RANK to RANKL (OPG-L, osteoclast differentation factor, ODF) and thus inhibits the recruitment, proliferation and activation of osteoclasts.

OPG shows an inhibitory effect on osteoclasts. Osteoclast formation activity may be determined principally by the relative concentration of OPG-L/osteoclast differentiation factor (ODF) to OPG/OCIF in the bone marrow microenviroment. Alterations of this ratio may be the major cause of bone loss in many imbalances in bone metabolism such as osteoporosis, osteopetrosis, metastatic osteolytic lesions and rheumatic bone degradation.

Possible research areas

- Postmenopausal and senile osteoporosis
- Glucocorticoid-induced osteoporosis
- Diseases with locally increased resorption activity
- Therapy monitoring after treatment with OPG
- Arthritis
- Oncology

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR1020	PLATE	Microtiter plate	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
KR1020	COATBUF	Coating buffer, ready-to-use	30 ml
KR1020	COATAB	Capture antibody (rat anti-mouse OPG), lyophilised	1 vial
KR1020	2. AB	Detection antibody (goat anti-mouse OPG, biotinylated), lyophilised	2 x 1 vial
KR1020	STD	Calibrator (4 000 pg), lyophilised	2 vials
KR1020	CONJ	Conjugate, (Strepdavidin-HRP-labelled), ready-to-use	1 vial
KR1020	DIL	Reagent diluent, ready-to-use	100 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised capture antibody (COATAB)** can be used until the expiry date stated on the label when stored at **2–8 °C**. Before use, the COATAB has to be reconstituted with **70 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **The capture antibody** (reconstituted COATAB) **can be stored at 2–8 °C for up to 60 days** or aliquoted and stored at **-20 °C to -70 °C for up to 6 months.**

  The **capture antibody** (reconstituted COATAB) contains 720 µg/ml of rat anti-mouse OPG. Dilute the **capture antibody** immediately before use to a **working concentration of 4 µg/ml in coating buffer** (COATBUF).
- The **lyophilised detection antibody (2. AB)** can be used until the expiry date stated on the label when stored at **2–8 °C**. Before use, the 2. AB has to be reconstituted with **70 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **The detection antibody** (reconstituted 2. AB) **can be stored at 2–8 °C for up to 60 days** or aliquoted and stored at **-20 °C to -70 °C for up to 6 months.**

  The **detection antibody** (reconstituted 2. AB) contains 36 µg/ml of biotinylated goat anti-mouse OPG. Dilute the **detection antibody** immediately before use to a **working concentration of 200 ng/ml in reagent diluent** (DIL).
- The **lyophilised standard (STD)** can be used until the expiry date stated on the label when stored at **2–8 °C**. Before use, the STD has to be reconstitut-

ed with **600 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 15 minutes and then mix thoroughly. The **standard** (reconstituted STD) **can be stored at 2–8 °C overnight or aliquoted and stored at -70 °C for up to 2 months.** A seven point standard curve using 2-fold serial dilutions in reagent diluent (DIL), and a high standard of 4000 pg/ml is recommended.

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:200** in **reagent dilution** (DIL). The **CONJ** can be used until the expiry date stated on the label when stored at **2–8 °C**. **Conjugate** (1:200 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents can be used until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF 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.

7. ASSAY PROCEDURE

Principle of the test

This sandwich ELISA is an assay for the direct determination of OPG in serum, plasma and urine. Two highly specific antibodies against OPG 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 OPG react with the coated capture antibody on the microtiter plate. A sandwich-type complex is formed consisting of the binding antibody on the plate, OPG 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, tetramethylbenzidine. An acidic stopping solution is subsequently added. The blue colour changes to yellow. The intensity of the yellow colour is directly proportional to the concentration of OPG in the sample.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. OPG, present in the samples, is determined directly from this curve.

Plate preparation

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2–8 °C. Strips can be used until expiry date stated on the label.

1.	Coat a 96-well microplate with each **100 µl** of the **working concentration of the capture antibody**. Seal the plate and incubate **overnight** at room temperature.
2.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3.	Block the wells by adding **250 µl reagent diluent** (DIL) into each well. Incubate at room temperature for **1 hour**.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper. The plates are now ready for sample addition.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/samples on a protocol sheet.

We recommend to carry out the tests in duplicate.

5.	Add **100 µl of sample or standard** into each well.
6.	Cover the strips and incubate for **2 h** at room temperature (15–30 °C).
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl** of the **working dilution of the detection antibody** into each well.
9.	Cover the strips and incubate for **2 h** at room temperature (15–30 °C).

10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add **100 µl** of the **working dilution of the conjugate** into each well.
12.	Cover the strips and incubate for **20 min** at room temperature (15–30 °C). Avoid placing the plate in direct light.
13.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
14.	Add **100 µl substrate** (SUB) into each well.
15.	Incubate for **10–20 min*** at room temperature (15–30 °C) in the **dark**.
16.	Add **50 µl stop solution** (STOP) into each well. Gently tap the plate to ensure thorough mixing.
17.	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.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

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

3. Spline algorithm

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

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

9. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted in wash buffer and re-assayed. Please consider this higher dilution when calculating the results.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

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

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for 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 incorrect use.
- Warranty claims and complaints regarding 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.

15. REFERENCES

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4. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C et al., Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. (1998) *Genes and Development* **12**: 1260- 1268
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7. Honore P et al., Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. *Nature Medicine* (2000), **6**(5): 521-528

Used symbols:


Temperature limitation

Catalogue Number

For research use only

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet