Osteoprotegerin ELISA

Manufactured by Immundiagnostik AG.

ALX-850-280A-KI01

(Version: 2019-09-05)

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For laboratory use only. Not for human or diagnostic use.

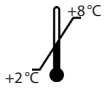
Manual

Osteoprotegerin ELISA

For the in vitro determination of OPG in serum and plasma

Gültig ab / Valid from: 2019-09-05

REF **K 1011**



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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of osteoprotegerin in serum, EDTA plasma, heparin plasma or citrate plasma. For *in vitro* diagnostic use only.

2. INTRODUCTION

Osteoprotegerin (OPG) or osteoclast inhibitory factor (OCIF) is a glycoprotein of the TNF receptor superfamily 11b (gene name TNFRSF11B) http://www.uniprot.org/uniprot/O00300. OPG is synthesised as a monomer of 380 amino acids and is assembled as a homodimer within the cell, and then secreted mainly as a disulfide-linked homodimer into the extracellular compartment. OPG is produced by many different tissues and cell types including osteoblasts. OPG is a negative regulator of bone resorption by acting as decoy receptor for RANKL, thus neutralising its function in osteoclastogenesis. This glycoprotein is also involved in the regulation of vascular calcification.

Indications

- Osteoporosis [1, 2]
- Diseases with locally incr. resorption activity [3 - 6]
- Arthritis [7, 8]
- Therapy monitoring [9 - 11]
- Cardiovascular Disease [12 - 17]

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 1011	PLATE	Microtiter plate, pre-coated (goat anti OPG, polyclonal)	12 x 8 wells
K 1011	WASHBUF	Wash buffer concentrate, 20 x	1 x 50 ml
K 1011	AB	Detection antibody, ready-to-use (mouse anti OPG, monoclonal)	1 x 7 ml
K 1011	CONJ	Conjugate, ready-to-use (peroxidase-labelled)	1 x 22 ml
K 1011	STD	Standards, ready-to-use (0; 1.25; 2.5; 5; 10; 20 pmol/l)	6 x 300 µl
K 1011	CTRL	Control, ready-to-use (see specification for range)	1 x 300 µl

Cat. No.	Label	Kit components	Quantity
K 1011	ASYBUF	Assay buffer, ready-to-use	1 x 25 ml
K 1011	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 22 ml
K 1011	STOP	Stop solution, ready-to-use	1 x 7 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
- Plate washer is recommended for washing, alternatively multi-channel pipets or repeater pipets
- Centrifuge, 3000 *g*
- 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. PREPARATION AND STORAGE OF REAGENTS

- 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 **1:20** before use (50 ml WASHBUF + 950 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** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:20 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

- Collect venous blood samples by using standardised blood collection tubes for serum or plasma. We recommend performing plasma or serum separation by centrifugation as soon as possible (e. g. 20 min at 2000 *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 (up to one day).
- The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -20 °C or lower. Samples are at least stable for 4 freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results.
- Samples should be mixed well before assaying.

7. ASSAY PROCEDURE

Principle of the test

The assay utilises the "sandwich" technique with specific antibodies against OPG. Standards, controls and samples which are assayed for OPG are added into the wells of a microtiterplate coated with polyclonal goat-anti-OPG-antibody. During the first incubation step, OPG is bound by the immobilised primary antibody. Then a biotinylated monoclonal mouse-anti-OPG-antibody is added into each microtiter well. In the next step, the streptavidin-peroxidase-conjugate is added and a "sandwich" of

1st antibody – OPG - biotinylated antibody – streptavidin-peroxidase-conjugate

is formed. Tetramethylbenzidine is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of OPG. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. standard concentration is generated, using the values obtained from the standard. OPG, present in the patient samples, is determined directly from this curve.

Test procedure

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

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

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Add **150 µl assay buffer** (ASYBUF) into each well. Add further **100 µl assay buffer** in the well for the **blank**.
2.	Add each **20 µl standards/controls/samples** into the respective wells.
3.	Add **50 µl detection antibody** (AB) into each well, **except BLANK**, mix gently.
4.	Cover the strips tightly and incubate for **4h** at room temperature (15–30 °C).
5.	Discard the content of each well and wash **5 times** with **300 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
6.	Add **200 µl conjugate** (CONJ) into each well.
7.	Cover tightly and incubate for **1 hour** at room temperature (15–30 °C).
8.	Discard the content of each well and wash **5 times** with **300 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
9.	Add **200 µl substrate** (SUB) into each well.
10.	Incubate for **30 min*** at room temperature (15–30 °C) in the **dark**.
11.	Add **50 µl stop solution** (STOP) into each well and mix well.
12.	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 an OD higher than the OD of the highest standard can be further diluted with STD 1 or OPG negative human serum 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 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.

Reference range

Based on a laboratory intern study of samples of apparently healthy persons (n = 60), a median of 2,7 pmol/ml was estimated.

We recommend each laboratory to establish its own reference range.

Conversion factor

1 pg/ml = 0.05 pmol/l

OPG-data from Kudlacek et al. 2003

OPG reference data[8] (n = 1134, 50.83 ± 51.47 pg/ml, median 36, 2-584;
(n = 1134, 2.54 ± 2.57 pmol/L, median 1.8, 0.1-29.2)

Age	OPG (mean ± SD)		OPG (median, range)		n
	pg/ml	pmol/L	pg/ml	pmol/L	
Female population (n = 687, 51.9 ± 51.3 pg/ml, median 39; 2.60 ± 2.57 pmol/L, median 1.95)					
< 30	44.5 ± 21.2	2.23 ± 1.06	41.7, 87.7	2.09, 4.39	48
31-40	44.1 ± 1.25	2.21 ± 1.69	36, 260	1.8, 13	198
41-50	41.2 ± 25.0	2.06 ± 33.8	36, 204	1.8, 10.2	217
51-60	39.5 ± 22.3	1.98 ± 1.12	35.5, 160	1.78, 8	150
61-70	69.6 ± 62.9	3.48 ± 3.15	60.5, 342	3.03, 17.1	34
71-80	134.0 ± 70.0	6.70 ± 3.50	131, 244	6.55, 12.2	16
> 81	227.0 ± 100.0	11.35 ± 5.00	206, 479	10.3, 23.95	24
Male Population (n = 447, 50 ± 51.7 pg/ml, median 34.8; 2.5 ± 2.59 pmol/L, median 1.74)					
< 30	40.7 ± 26.3	2.04 ± 1.32	32, 96	1.6, 4.8	19
31-40	41.4 ± 30.5	2.07 ± 1.53	33.5, 170	1.68, 8.5	72
41-50	36.3 ± 31.8	1.82 ± 1.59	36.2, 316	1.81, 15.8	116
51-60	36.9 ± 20.2	1.85 ± 1.01	34, 134	1.7, 6.7	176
61-70	41.0 ± 22.7	2.05 ± 1.14	29.8, 160	1.49, 8	32
71-80	119.0 ± 71.0	5.95 ± 3.55	38, 103	1.9, 5.15	12
> 81	226.0 ± 78.0	11.30 ± 3.90	130, 219	6.5, 10.95	20

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 5

Sample	Mean [pmol/l]	CV [%]	Standard deviation
1	3.2	2	0.05
2	10.1	3	0.34

Inter-Assay (n = 12)

Sample	Mean [pmol/l]	CV [%]	Standard deviation
1	3.2	3	0.10
2	9.9	5	0.50

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- 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.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color 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 must 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 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

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The 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 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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Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> test

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet