

OSTEOPROTEGERIN

Enzyme immunoassay for the quantitative determination
of Osteoprotegerin in biological fluids

Cat. No. BI-20402

12 x 8 tests

For research use only!

BIOMEDICA

BIOMEDICA
GRUPPE 

Biomedica Medizinprodukte
Gesellschaft mbH & Co KG
A-1210 Wien, Divischgasse 4
Tel. +43/1/291 07 50
Fax +43/1/291 07 71
exp.biomedica@bmgrp.at

www.biomedica.co.at

Contents

	Page
1.) Introduction	3
2.) Principle of the assay	3
3.) Contents of the kit	4
4.) Additional material and equipment required	4
5.) Reagents and sample preparation - assay procedure	4
6.) Calculation of results	6
7.) Assay characteristics	6
8.) Technical hints	6
9.) Precautions	7
10.) References	7
11.) Incubation scheme	8

1) Introduction

The morphogenesis and remodeling of bone requires the synthesis of bone matrix by osteoblasts and its coordinated resorption by osteoclasts. Osteoprotegerin (OPG) also known as Osteoclast Inhibiting Factor (OCIF) or Osteoclast Binding Factor (OBF), is a key factor inhibiting the differentiation and activation of osteoclasts, and is therefore essential for bone resorption. Osteoprotegerin is a glycoprotein belonging to the TNF receptor family. As a so called soluble "decoy" receptor, Osteoprotegerin inhibits the binding of RANK to RANKL and thus inhibits the recruitment, proliferation and activation of osteoclasts.

In adult humans OPG/OCIF mRNA is highly expressed in various tissues e.g. heart, lung, kidneys, bone, liver, placenta, brain.

Abnormalities in the balance of sRANKL/RANK/OPG system lead to severe disturbances of bone remodeling that underlie the bone damage of postmenopausal osteoporosis, Paget's disease, bone loss in metastatic cancers and rheumatoid arthritis.

Possible Indications:

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

2.) Principle of the assay

The Osteoprotegerin test kit is an enzyme immunoassay designed to determine Osteoprotegerin directly in biological fluids (serum, plasma, cell culture supernatants). In a first step, assay buffer, sample and the biotinylated OPG detection antibody are added simultaneously to the wells. Osteoprotegerin, if present in the sample, binds to the precoated capture antibody and forms a sandwich with the detection antibody.

After a washing step, which removes all non-specific bound material, streptavidin-HRP conjugate is added to the wells. After removal of unbound conjugate by washing, Tetramethylbenzidine (TMB) is added to the wells as substrate. Osteoprotegerin is quantitated by an enzyme catalysed colour change detectable on a standard ELISA reader. The amount of colour developed is directly proportional to the amount of Osteoprotegerin present in the sample.

3.) Contents of the kit

- 12 x 8 well microtiter strips in strip holder, packed in alubag with desiccant. Wells are coated with a monoclonal anti-Osteoprotegerin antibody
- 10x washing buffer; concentrated
The bottle contains 100 ml of washing buffer concentrate
- 5 standards ranging from 0 to 30 pmol/l (white caps) The vial contains synthetic human Osteoprotegerin in human serum base, ready for use. The exact concentrations are stated on the label.
- One human serum base control, ready for use. The exact concentration is stated on the label.
- Detection antibody (glass vial, yellow solution)
The vial contains biotinylated polyclonal anti-Osteoprotegerin antibody, sufficient for 96 determinations, ready for use.
- Conjugate
The vial contains 22 ml streptavidin-HRP conjugate, ready for use.
- Substrate
The vial contains 22 ml TMB solution, ready for use
- Stop solution
The vial contains 7 ml of stop solution, ready for use
- Assay buffer
The bottle contains 25 ml of assay buffer.
- Osteoprotegerin stock
The vial contains Osteoprotegerin stock solution, 500 pmol/l.
- 2 self-adhesive plastic films
- Protocol sheet
- Instructions for use (package insert)

4.) Additional material and equipment required

Distilled water

Variable volume pipettes in the range of 50 µl to 1000 µl

Multichannel or multipipette

ELISA reader equipped with 450 nm filter

Graph paper or software for calculation of results

5.) Reagents and sample preparation - assay procedure

Store samples at -20°C if not assayed on the same day. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying.

We recommend duplicates for all values.

- Dilute washing buffer concentrate to 1000 ml (add the 100 ml of concentrate to 900 ml of distilled water) and mix well. Crystals in the buffer concentrate will dissolve at room temperature. Buffer is stable at 4°C until expiry date stated on the label.
1. Mark positions for blank, standards, control, and samples on the protocol sheet supplied.
 2. Take microtiter strips out of the alu bag and mark as appropriate. Mark a minimum of 1 well as blank. Store unused strips with the desiccant at 4°C in the alu bag supplied. Strips are stable until expiry date stated on the label.
 3. Pipette 100 µl of assaybuffer into each well. Pipette additional 100 µl into well marked as blank.
 4. Pipette 50 µl of standards, control or samples in the respective wells.
 5. Add 50 µl detection antibody (glass vial, yellow solution) to all wells, except blank. Mix gently.
 6. Cover strips with plastic film and incubate overnight (16 - 24 hours) at 4°C. Make sure all wells are sealed well with the plastic film to avoid evaporation.
 7. Discard contents of the wells and wash 5x with minimum 300 µl diluted wash buffer. Remove any remaining fluid by hitting plate against paper towel after the last wash.
 8. Add 200 µl conjugate (red solution) to all wells and cover strips with plastic film.
 9. Incubate for 60 min. at room temperature (18-26°C). Mix gently.
 10. Discard contents of the wells and wash 5x with minimum 300 µl diluted wash buffer. Remove any remaining fluid by hitting plate against paper towel after the last wash.
 11. Add 200µl of substrate to all wells and incubate for 20 min. at room temperature (18-26°C) in the dark.
 12. Add 50 µl stop solution to all wells, mix gently.
 13. Determine absorption immediately with an ELISA reader at 450 nm against 690 or 620 nm as reference.
If the correction wavelength of 620 or 690 nm is not available read only at 450nm.
 14. If the extinction of the highest standard exceeds the measuring range of the photometer, absorption can be measured immediately at 405 nm against 690 or 620 nm as reference.
 15. When concentrations of more than 30 pmol/l are expected, samples must be diluted with assay buffer and assayed again. The dilution factor must be considered in calculating the results.

Measurement of Osteoprotegerin in Cell Culture Supernatants:

An Osteoprotegerin stock is added to the kit containing 500 pmol/l.

Prepare a serial dilution of the Osteoprotegerin stock solution with cell culture medium, according to your needs. Cell culture medium is used as a 0-standard.

If necessary, dilute cell culture supernatant according to the expected concentration with the cell culture medium. Dilution of the supernatant is dependent on amount of Osteoprotegerin secreted by the respective cell type.

The shape of the calibration curve may vary depending on the composition of the culture medium used. Therefore a 4 PL algorithm is recommended. If cell culture supernatants are diluted, the dilution factor must be considered in calculating the results.

6.) Calculation of results

The extinction of the blank is subtracted from all other values. A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.

The assay has been evaluated using a 4 PL algorithm. Different curve fitting methods need to be evaluated by the user.

Respective dilution factors have to be considered.

7.) Assay characteristics

Normal range: It is recommended to establish the normal range for each laboratory.

Standard range: 0 to 30 pmol/l

Conversion factor pg/ml to pmol/l: 1 pg/ml = 0.05 pmol/l (MW: 19.9 kD)

Sample volume: 50 µl human EDTA plasma, Heparin plasma, serum, cell culture supernatant

Detection Limit: (0 pmol/l + 3 SD): 0.14 pmol/l

Incubation time: overnight / 1h / 20 min

Precision:

	4,6 pmol/l	19,6 pmol/l
Intra-Assay (n=16)	< 10 %	< 10 %
Inter-Assay (n=16)	< 10 %	< 10 %

Storage: 4°C

8.) Technical hints

- To avoid cross-contamination, change pipette tips between addition of standards, controls, samples, antibody, conjugate and substrate. Also use separate reservoirs for each reagent.
- When mixing reagents, always avoid foaming.
- 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.

9.) Precautions

All liquid reagents contain 0.01% Thimerosal or 0,01% Proclin 300 as preservative.

- Thimerosal is toxic! 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.
- Do not interchange kit components from different lots.
- Do not use kit components beyond the expiry date.
- 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 contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

The OPG Elisa was developed in cooperation with Immundiagnostik, Bensheim, Germany
--

10.)References

Literature:

- 1) Hofbauer L.C., Osteoprotegerin ligand and osteoprotegerin: novel implications for osteoclast biology and bone metabolism. *European Journal of Endocrinology* (1999), 141: 195-210
- 2) Hofbauer L.C. & A.E. Heufelder, The Role of Osteoprotegerin and Receptor Activator of Nuclear Factor KB Ligand in the Pathogenesis and Treatment of Rheumatoid Arthritis. *Arthritis & Rheumatism* (2001), 44:253-259
- 3) Bucay N. et al., Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes and Development* (1998), 12:1260-1268
- 4) Bekker P.J. et al., The Effect of a Single Dose of Osteoprotegerin in Postmenopausal Women. *Journal of Bone and Mineral Research* (2001), 16:348-360
- 5) Makhluif H.A., Age-Related Decline in Osteoprotegerin Expression by Human Bone Marrow Cells Cultured in Three-Dimensional Collagen Sponges. *Biochemical and Biophysical Research Communications* (2000), 268: 669-672
- 6) 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.
- 7) Warren S. et al., Associations of Serum Osteoprotegerin Levels with Diabetes, Stroke, Bone Density, Fractures and Mortality in Elderly Women. *Journal of Clinical Endocrinology & Metabolism* (2001), 86: 631-637
- 8) Aubin J.E. & E. Bonnelye, Osteoprotegerin and its Ligand: A New Paradigm for Regulation of Osteoclastogenesis and Bone Resorption. *Medscape Women Health* (2000), 5(2)

11.)Incubation scheme

Sample incubation

	Blank	Standard/Control	Sample
Assay buffer	200µl	100µl	100µl
Standard/Control	-	50µl	-
Sample	-	-	50 µl
Detection antibody	-	50µl	50 µl

Cover strips with the supplied plastic film and incubate overnight (16-24h) at 4°C.
Discard the content of the wells and wash 5 times with 300 µl diluted wash buffer.
Remove any remaining fluid by hitting plate against paper towel after the last wash.

Conjugate incubation

Conjugate	200 µl	200 µl	200 µl
-----------	--------	--------	--------

Cover strips with the supplied plastic foil and incubate for 1 hour at room temperature (18-26°C)
Discard the content of the wells and wash 5 times with 300 µl diluted wash buffer.
Remove any remaining fluid by hitting plate against paper towel after the last wash.

Substrate incubation

Substrate	200 µl	200 µl	200 µl
-----------	--------	--------	--------

Incubate 20 min. at room temperature (18-26°C) in the dark.

Stop and read

Stop solution	50 µl	50 µl	50 µl
---------------	-------	-------	-------

Mix, and read absorption with an ELISA microwell reader at 450 nm and 620 nm or 690 nm as a reference. If the correction wavelength of 620 or 690 nm is not available read only at 450 nm. If the readings exceed the measuring range of the photometer, absorption can be measured at 405 nm against 690 nm or 620 nm as reference immediately.

Vienna, May 2001