

# ***OSTEOPROTEGERIN***

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF OSTEOPROTEGERIN IN SERUM, EDTA PLASMA, HEPARIN  
PLASMA OR CITRATE PLASMA  
CAT. NO. BI-20403 . 12 X 8 TESTS

FOR RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES

## ***CONTENT***

Additional information on our products is available on our website.

***[www.bmgrp.com](http://www.bmgrp.com)***

## 1) 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 synthesized 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 neutralizing its function in osteoclastogenesis. This glycoprotein is also involved in the regulation of vascular calcification.

### Areas of interest:

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

## 2) CONTENT OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Goat polyclonal anti OPG antibody, pre-coated microtiter strips in a stripholder, packed in an aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
AB	Mouse monoclonal anti OPG antibody, biotin labelled, green cap, yellow dye, ready to use	1 x 7 ml
STD	Standards 1-6, (0; 1.25; 2.5; 5; 10; 20 pmol/l), white caps, ready to use	6 x 300 µl
CTRL	Control, yellow cap, ready to use, (exact concentration on the label)	1 x 300 µl
ASYBUF	Assay Buffer, red cap, ready to use	1 x 25 ml
CONJ	Conjugate, (streptavidin-HRPO), amber bottle, 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

## 3) ADDITIONAL MATERIAL IN THE KIT

- 2 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

## 4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 20 µl, 50 µl, 150 µl, 200 µl, 300 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

## 5) REAGENTS AND SAMPLE PREPARATION

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

### Sample preparation:

Collect venous blood samples by using standardized 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 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 (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 -25°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. Samples with values above highest STD can be diluted with STD1 or OPG negative human serum.

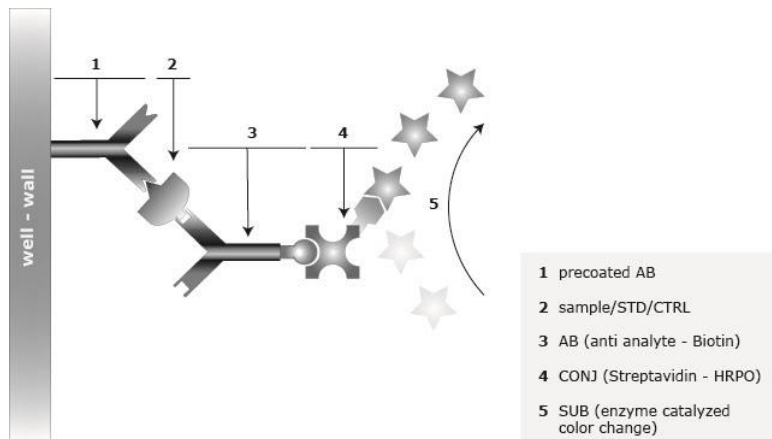
For further information on sample stability please visit our website [www.bmgrp.com](http://www.bmgrp.com) (see Validation Data) or contact our customer service by e-mail [export@bmgrp.com](mailto:export@bmgrp.com) or by phone +43/ 1/ 29107-45.

### Reconstitution/Handling:

**WASHBUF (Wash buffer):** Salt precipitate in the concentrated wash buffer is normal. Dissolve any precipitate by mixing gently at room temperature then dilute the concentrate 1:20 with distilled/DI water, e.g. 50 ml WASHBUF + 950 ml distilled water, prior to using in the assay. Undiluted wash buffer is stable at 4°C (2-8°C) until expiry date on the label. Diluted wash buffer is stable at 4°C (2-8°C) for one month. Only use diluted WASHBUF (Wash buffer) for optimum assay performance.

## 6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the determination of OPG in human serum and plasma samples. In a first step, assay buffer, STD/sample/CTRL, and detection antibody (mouse monoclonal anti human OPG-Biotin) are pipetted into the wells of the microtiter strips, which are pre-coated with goat polyclonal anti OPG antibody. OPG present in the STD/sample/CTRL binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. In the washing step all non-specific unbound material is removed. In a second step, the conjugate (Streptavidin-HRPO) is pipetted into the wells and reacts with the detection antibody. After another washing step, the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of OPG. This colour change is detectable with a standard microtiter plate ELISA reader. A dose response curve of the absorbance (optical density, OD at 450 nm) vs. standard concentration is generated, using the values obtained from the standard. The concentration of OPG in the sample is determined directly from the dose response curve.



## 7) ASSAY PROTOCOL

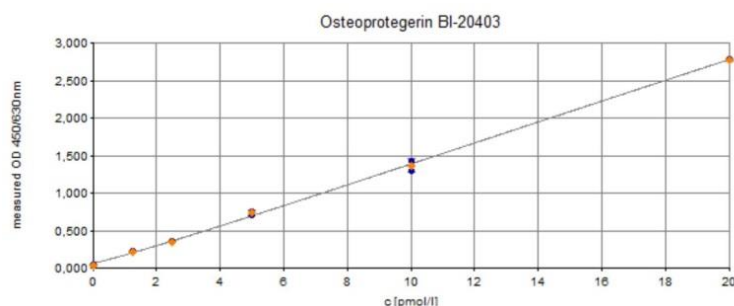
All reagents and samples must be at room temperature (18-24°C) before use in the assay.  
 Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.  
 Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

- 1) Pipette 150 µl ASYBUF (Assay Buffer, red cap) into each well.
- 2) Add 20 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells, swirl gently.
- 3) Add 50 µl AB (biotinylated anti OPG antibody, green cap) into each well, swirl gently.
- 4) **Cover tightly and incubate for 4 hours at room temperature (18-24°C).**
- 5) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- 6) Add 200 µl CONJ (Conjugate, amber cap) into each well.
- 7) **Cover tightly and incubate for 1 hour at room temperature (18-24°C).**
- 8) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- 9) Add 200 µl SUB (Substrate, blue cap) into each well.
- 10) **Incubate for 30 min at room temperature (18-24°C) in the dark.**
- 11) Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
- 12) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

## 8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit at production date. Data for OD 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 STD6 (standard with highest concentration) and the value of the CTRL is in range (target range see label).

## 9) ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips
Sample type:	Serum, EDTA plasma, heparin plasma, and citrate plasma
Standard range:	0 to 20 pmol/l (6 standards and 1 control in a human serum matrix)
Conversion factor:	1 pg/ml = 0.05 pmol/l (MW: 19.9 kD)
Sample volume:	20 µl / well
Incubation time:	4 h / 1 h / 30 min
Sensitivity:	LOD: (0 pmol/l + 3 SD): 0.07 pmol/l; LLOQ: 0.08 pmol/l
Specificity:	The ELISA recognizes human endogenous and recombinant OPG. The OPG ELISA detects monomeric and dimeric OPG as well as OPG-RANKL complexes.

	The assay does not cross-react with rat or mouse samples.	
Precision:	Intra-assay (n=5) ≤ 3%, Inter-assay (n=12) ≤ 5%	
Spike/Recovery (average recovery spiked with rec. OPG):	Serum (n=3): 98%	Heparin plasma (n=3): 89%
	EDTA plasma (n=3): 100%	Citrate plasma (n=3): 95%
Dilution linearity (average recovery of expected OPG after a 1+1; 1+3; 1+7 dilution):	Dilution:	Serum (n=3)
	1 + 1	98%
	1 + 3	90%
	1 + 7	87%
Values from apparently healthy individuals:	Median serum (n=60): 2.7 pmol/l Median EDTA plasma (n=6): 2.2 pmol/l Median heparin plasma (n=7): 2.3 pmol/l Median citrate plasma (n=5): 2.3 pmol/l  Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.	

For further information on assay characteristics please visit our website [www.bmgrp.com](http://www.bmgrp.com) (see Validation Data) or contact our customer service by e-mail [export@bmgrp.com](mailto:export@bmgrp.com) or by phone +43/ 1/ 29107-45.

#### 10) PRECISION

Intra-assay: 2 samples of known concentrations were tested 5 times.

Inter-assay: 2 samples of known concentrations were tested 12 times in 2 different kit lots by 3 different operators.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=12)	Sample 1	Sample 2
Mean (pmol/l)	3.2	10.1	Mean (pmol/l)	3.2	9.9
SD (pmol/l)	0.05	0.34	SD (pmol/l)	0.10	0.50
CV (%)	2	3	CV (%)	3	5

#### 11) TECHNICAL HINTS

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

#### 12) 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.

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.

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

### 13) LITERATURE

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6. Pepene C et al.: Circulating osteoprotegerin and soluble receptor activator of nuclear factor B ligand in polycystic ovary syndrome: relationships to insulin resistance and endothelial dysfunction. *Eur J Endocrinol* (2011), 164: 61-68.
7. Tuyl van L.: Baseline RANKL:OPG ratio and markers of bone and cartilage degradation predict annual radiological progression over 11 years in rheumatoid arthritis. *Ann Rheum Dis* (2010), 69: 1623-1628.
8. Anastasilakis AD et al.: Evaluation of Bone Mineral Density, Bone Metabolism, Osteoprotegerin and Receptor Activator of the NF $\kappa$ B Ligand Serum Levels During Treatment with Infliximab in Patients with Rheumatoid Arthritis. *Eur J Endocrinol* (2008), 158: 411-415.
9. Faienza F et al.: Osteoclastogenesis in Children with 21-Hydroxylase Deficiency on Long-Term Glucocorticoid Therapy: The Role of Receptor Activator of Nuclear Factor- $\kappa$ B Ligand/Osteoprotegerin Imbalance. *J Clin Endocrinol Metab* (2009), 94: 2269-2276.
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12. Semb A et al.: Osteoprotegerin and Soluble Receptor Activator of Nuclear Factor- $\kappa$ B Ligand and Risk for Coronary Events. A Nested Case-Control Approach in the Prospective EPIC-Norfolk Population Study. 1993-2003. *Arterioscler Thromb Vasc Biol* (2009), 29: 975-980.
13. Svensson M et al.: Osteoprotegerin as a predictor of renal and cardiovascular outcomes in renal transplant recipients: follow-up data from the ALERT study. *Nephrol Dial Transplant* (2011), 10.1093/ndt/gfr694.
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15. Lieb W et al.: Biomarkers of the Osteoprotegerin Pathway: Clinical Correlates, Subclinical Disease, Incident Cardiovascular Disease, and Mortality. *Arterioscler Thromb Vasc Biol* (2010): 30: 1849-1854.
16. Ueland T et al.: Osteoprotegerin Predicts Progression of Chronic Heart Failure: Results From CORONA. *Circ Heart Fail* (2011): 4: 145-152.
17. Jiang JQ et al.: Serum osteoprotegerin measurement for early diagnosis of chronic kidney disease-mineral and bone disorder. *Nephrology* (2011), 16(6): 588-594.

## SYMBOLS



Expiry date / Verfallsdatum / Date de péremption / Data di scadenza / Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Utløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba expirácie / Doba expirace



Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití



In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use) / In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícký materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnícký materiál (určeno pro diagnostiku „in vitro“)



Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže



Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno



Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencennummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalógové číslo



Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk ..... között / Skladujte v rozsahu / Skladujte v rozmezí



Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehåller räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

# **BI-20403 OSTEOPROTEGERIN**

## **ASSAY PROTOCOL AND CHECKLIST**

### **PREPARATION OF REAGENTS:**

- Bring all reagents to room temperature (18-24°C).
- Prepare reagents and samples as instructed.
- Bring unused and prepared components to the storage temperature mentioned in the package insert.
- Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

### **TEST PROCEDURE:**

- Step 1) Pipette 150 µl ASYBUF (Assay buffer, red cap) into each well.
- Step 2) Add 20 µl STD/SAMPLE/CTRL (standard/sample/control) into all wells, swirl gently.
- Step 3) Add 50 µl AB (biotinylated anti OPG ab, green cap) into each well, swirl gently.
- Step 4) Cover tightly and incubate for 4 h at room temperature (18-24°C).**
- Step 5) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Step 6) Add 200 µl CONJ (Conjugate, amber cap) into each well.
- Step 7) Cover tightly and incubate for 1 h at room temperature (18-24°C).**
- Step 8) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- Step 9) Add 200 µl SUB (Substrate, blue cap) into each well.
- Step 10) Incubate for 30 minutes at room temperature (18-24°C), in the dark.**
- Step 11) Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
- Step 12) Read Optical Density at 450 nm with reference 630 nm, if available.

## Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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*For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*

