DRG® Osteoprotegerin (OPG) (EIA-3551)

CE

RUO in the USA

Revised 24 April 2007

INTENDED USE

The DRG assay is a sandwich ELISA intended for the quantitative determination of Osteoprotegerin in serum, plasma, urine and cell culture supernatants.
It is for in vitro use only. In the United States, this kit is intended for Research Use Only.

SUMMARY AND EXPLANATION OF THE TEST

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.

Indication

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

PRINCIPLE OF THE TEST

This sandwich-type ELISA is an assay for the direct determination of OPG in serum, plasma and urine. In this assay two highly specific antibodies against OPG are used. The binding 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 simultaneously with the pre-coated antibody on the microtiterplate. A sandwich-type complex is found 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, TMB. 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 units (at 450 nm) versus concentration is generated. OPG, present in the samples, is determined directly from this calibration curve.

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MATERIAL SUPPLIED

Content	Kit Components	Quantity
Micro wells	Pre-coated microtiterplate, 12 x 8 strips	96
Wash Buffer	ELISA washing buffer concentrate (20x)	50 ml
Assay Buffer	Assay buffer, ready-to-use	25 ml
Standard	Calibrators, ready-to-use (0; 0.37; 1.1; 3.3; 10; 30 pmol/l)	6 x 500 µl
Stock Solution	Stock solution (500 pmol/l)	500 µl
Control	Control, ready-to-use	500 µl
Antibody	2nd antibody (anti-OPG, biotinylated), ready-to-use	7 ml
Conjugate	Conjugate, (streptavidin-HRP-labeled), ready-to-use	22 ml
TMB Substrate Solution	TMB substrate (Tetramethylbenzidine), ready-to-use	22 ml
Stop Solution	ELISA stop solution, ready-to-use	7 ml

MATERIAL REQUIRED BUT NOT SUPPLIED

- 1,5 ml reaction vials (Eppendorf)
- Precision pipettes calibrated to deliver 10 -1000 µl and disposable tips.
- Centrifuge capable of 3000 x g
- ELISA reader
- Vortex-mixer
- Bidistilled or deionized water

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.

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- The **Wash Buffer** (wash buffer concentrate) should be diluted with aqua bidist. **1:20** before use (50 ml concentrate + 950 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 **Wash Buffer** 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.**
- **Cell culture**
  The **Stock Solution** (OPG calibrator concentrate, 500 pmol/l) must be diluted **1:20** with medium (50 µl Calibrator + 950 µl Medium) to obtain **S1**.
  Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Standard curve solutions must be prepared from S1 in **1:2** dilution steps by adding dilution medium as follows:

		S1	**(25 pmol/l)**
200 µl S1	+ 200 µl dilution medium	= **S2**	**(12.5 pmol/l)**
200 µl S2	+ 200 µl dilution medium	= **S3**	**(6.25 pmol/l)**
200 µl S3	+ 200 µl dilution medium	= **S4**	**(3.13 pmol/l)**

Cell culture medium is used as Standard 0.

PRECAUTIONS

- For in vitro use only. In the United States, this kit is intended for Research 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.
- 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.
- Reagents should not be used beyond the expiration date stated on kit label.

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.
Lipton et al. (2002) Serum Osteoprotegerin Levels in Healthy Controls and Cancer Patients. Cancer Res. 8:2306-231 0

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. DRG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.

Test procedure

It is recommended to carry out the assay in duplicate and to cover the microtiter plate during the incubation steps.

1. Add 100 µl *Assay Buffer* into each well.
2. Add 50 µl *Standard, Control or samples* into each microtiter well induplicate.
3. Add 50 µl Antibody into each well. Mix gently.
4. Incubate **over night** (18 – 24 h) **at 2-8 °C**.
5. Aspirate and wash the wells 5 x with 300 µl ELISA wash buffer.
6. Add 200 µl Conjugate into each well.
7. Incubate for **1 hour at room temperature** (18 – 26 °C).
8. Aspirate and wash the wells 5 x with 300 µl ELISA wash buffer.
9. Add 200 µl TMB Substrate solution into each well.
10. Incubate for 15 – 20 min at room temperature in the dark.
11. Add 50 µl Stop Solution into each well and mix shortly.
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.

RESULTS

A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are calculated from this calibration curve.
THE CALIBRATION CURVE IS NOT LINEAR, therefore a 4PL- algorithm or spline is recommended.

LIMITATIONS

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

QUALITY CONTROL

DRG recommends to use control samples for internal quality control.

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values are located outside the acceptable limits, the results for the patient sample may not be valid.

Expected values

Reference values:
Normal sera measured with our OPG-ELISA gave the following values:

Healthy men (n=36)	1.715 pmol/l 1.747 pmol/l	(median) (mean ± 0.8254 SD)
Healthy women (n=32)	1.59 pmol/l 1.891 pmol/l	(median) (mean ± 1.206 SD)

Conversion factor: 1 pg/ml = 0.05 pmol/l
We recommend each laboratory to establish its own norm concentration range.

PERFORMANCE CHARACTERISTICS

Precision and reproducibility

The precision (intra-assay variation) of the DRG OPG ELISA test was calculated from 16 replicate determinations on each of one samples.
Table1: Intra-Assay CV (n= 16)

Sample	OPG [pmol/l]	Intra-Assay CV [%]
1	4,5	10
2	19,5	8

The total precision (inter-assay variation) was calculated from data on 2 samples obtained in 16 different assays by three technicians on two different lots of reagents over a period of three months.
Table 2: Inter-Assay CV (n= 16)

Sample	OPG [pmol/l]	Inter-Assay CV [%]
1	4,5	< 10
2	19,5	< 10

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Sensitivity

The detection limit (0 pg/ml + 3 SD): 0.14 pmol/l.

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for in vitro diagnostic use only. In the United States, this kit is intended for Research Use Only.
- Guidelines for medical laboratories should be followed.
- 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 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.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not mix different lot numbers of any kit component.
- 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. DRG 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 DRG along with a written complaint.

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SYMBOLS USED WITH DRG ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης

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	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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