Instructions for use

Osteocalcin (1-43/49) specific ELISA

Enzyme immunoassay for the in-vitro-diagnostic quantitative determination of Osteocalcin (1-43/49) in human serum and plasma.

REF **RE53141**

Σ **12x8**

2-8 °C

EU: IVD CE U.S.: *For research use only.* *Not for use in diagnostic procedures.*

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1. INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of both human osteocalcin (1-49) and osteocalcin (1-43) (also referred as N-terminal & mid-regional osteocalcin) levels in test samples. This test is useful for assessing the bone formation activity or osteoblast activity in patients associated with changes in the rate of bone turnover in metabolic bone disease, such as osteoporosis, primary hyperparathyroidism, hyperthyroidism, Paget's disease, and renal osteodystrophy.

2. SUMMARY AND EXPLANATION

Osteocalcin [also as bone Gla protein (BGP)] is a major noncollagenous protein found in bone and dentin. The synthesis of osteocalcin involves vitamin K and vitamin D3. Freshly synthesized osteocalcin is partly released into the blood stream and partly incorporated into the bone matrix. Both osteocalcin (1-49) and its fragments including osteocalcin (1-43) are released into the blood stream. Serum osteocalcin (1-43) also generated by catabolic breakdown of osteocalcin (1-49) in the circulation, liver, kidney, as well as by in vitro degradation during storage of samples, because a labile six-amino acid C-terminal sequence that, in vitro at room temperature, is easily cleaved off. There are several studies that have confirmed the measurement of the much more stable N-terminal and mid-regional osteocalcin [osteocalcin (1-43/49)] as being clinically useful, which may contribute to a more accurate assessment of the bone turnover rate.

As osteocalcin is manufactured by osteoblasts, it is often used as a biochemical marker, or biomarker, for the bone formation process. It has been routinely observed that higher serum-osteocalcin levels are relatively well correlated with increases in bone mineral density (BMD) during treatment with anabolic bone formation drugs for osteoporosis, such as Forteo. In many studies, Osteocalcin is used as a preliminary biomarker on the effectiveness of a given drug on bone formation.

3. TEST PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human osteocalcin (1-49) and (1-43) in serum or plasma sample. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human osteocalcin.

Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with streptavidin. Subsequently, a mixture of biotinylated human osteocalcin N-terminal region specific polyclonal antibody and a peroxidase labeled human osteocalcin 20 – 43 region specific monoclonal antibody is added to each well. After the first incubation period, a "sandwich" of "biotinylated antibody - human osteocalcin – HRP-monoclonal antibody" is formed and this immunocomplex is also captured to the wall of microtiter plate via a streptavidin-biotin affinity binding. The unbound monoclonal antibodies and buffer matrix are removed in the subsequent washing step. A substrate solution in a timed reaction is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human osteocalcin in a test sample. A standard curve is generated by plotting the absorbance versus the respective human osteocalcin concentration for each standard on point-to-point or 4 parameter curve fit. The concentration of human osteocalcin in test samples is determined directly from this standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.All components are stable until this expiration date.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.			
Only 50 µL of human serum or plasma sample is required for human osteocalcin measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube.			
Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	24 h	6 mon	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	**MTP**	**Microtiter Plate** Break apart strips. Coated with streptavidin.
1 x 6 x 0.5 mL	**CAL A-F** **LYO**	**Standard A-F**, lyophilized Contains: Human osteocalcin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. Refer to vials for exact concentration for each standard.
1 x 2 x 0.5 mL	**CONTROL 1 + 2** **LYO**	**Control 1 + 2**, lyophilized Contains: Human osteocalcin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. Refer to vials for exact concentration range for each control.
1 x 1.2 mL	**ENZCONJ** **CONC**	**HRP Conjugated Osteocalcin Antibody** One vial contains 1.2 mL HRP conjugated monoclonal anti-human osteocalcin (20- 43) antibody in a stabilized protein matrix. This reagent must be diluted with biotinylated antibody before use.
2 x 12 mL	**BIOTIN**	**Biotinylated Osteocalcin Antibody** Ready to use. Contains: Biotinylated anti-human osteocalcin N-terminal region specific antibody in a stabilized protein matrix. This reagent is ready to be used for dilution of HRP conjugated osteocalcin antibody.
1 x 30 mL	**WASHBUF** **CONC**	**Wash Buffer** Concentrate (30x) Contains: Phosphate buffered saline with a non-azide, non-mercury preservative.
1 x 22 mL	**TMB SUBS**	**TMB Substrate Solution** Ready to use. Tetramethylbenzidine (TMB) with hydrogen peroxide.
1 x 12 mL	**TMB STOP**	**TMB Stop Solution** Ready to use. 0.5 M H_2SO_4.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Serum or plasma sample collection tube.
2. Precision single channel pipettes capable of delivering 25 µL, 100 µL, 200 µL, and 1000 µL etc.
3. Repeating dispenser suitable for delivering 100 µL and 200 µL.
4. Disposable pipette tips suitable for above volume dispensing.
5. Disposable 12 x 75 mm or 13 x 100 plastic test tubes.
6. Disposable plastic 1000 mL bottle with cap.
7. Aluminum foil.
8. Deionized or distilled water.
9. Plastic microtiter well cover or polyethylene film.
10. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
11. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
12. ELISA plate shaker

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

⚠	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**

Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
400 µL	ENZCONJ CONC	with 8 mL	BIOTIN	1:21	Prepare freshly and use only once.		
10 mL	WASHBUF CONC	ad 300 mL	bidist. water	1:30		18-25°C	until Exp. date
	CAL A-F LYO	with 0.50 mL	bidist. water		Let stand for 15 min. Mix without foaming. One must make sure that all solid is dissolved completely prior to use.	≤ -20°C (Aliquots)	until Exp. date Do not exceed 3 freeze-thaw cycles.
	CONTROL 1+2 LYO	with 0.50 mL	bidist. water				

11. TEST PROCEDURE

1.	Pipette **25 µL** of each **Standard, Control and sample** into the respective wells of the Microtiter Plate.
2.	Pipette **200 µL** of **antibody mixture** (Biotin/Enzyme Conjugate) into each well.
3.	Cover plate with adhesive foil. **Incubate 60 min** at **RT (18-25°C) in the dark on an orbital shaker** (350 rpm ± 100 rpm).
4.	Remove adhesive foil. Discard incubation solution. Wash plate **5 x** with **350 - 400 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
6.	Pipette **200 µL** of **TMB Substrate Solution** into each well.
7.	**Incubate 20 min** at **RT (18-25°C) in the dark.**
8.	Stop the substrate reaction by adding **50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Colour changes from blue to yellow.
9.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **10 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

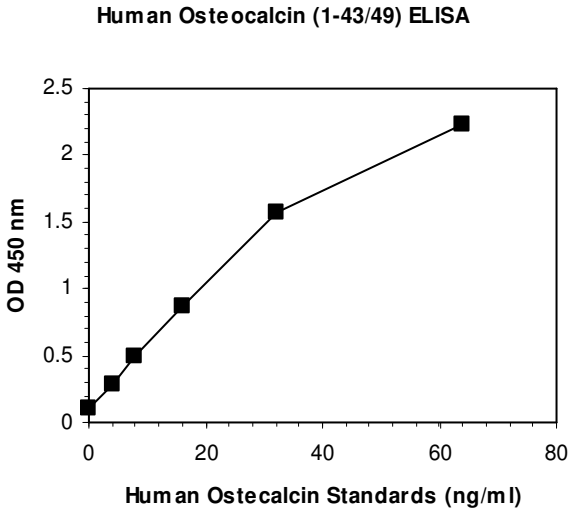
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the zero standard from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs (e.g. Point-to-Point, 4-Parameter) may also be used for the calculation of results.

The sample human osteocalcin concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Human Osteocalcin (ng/mL)	Mean OD
A	0	0.112
B	4	0.279
C	8	0.494
D	16	0.866
E	32	1.570
F	64	2.232

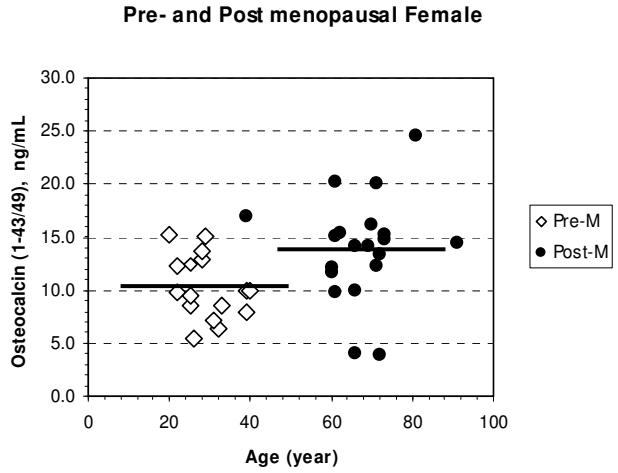


14. EXPECTED VALUES

Forty serum samples from normal healthy adults with age of 26 – 58 were collected and measured with this ELISA. The normal osteocalcin range was found to be 3.8 – 25.3 ng/mL and the mean osteocalcin level of this group was 11.7 ng/mL (median: 11.4 ng/mL) and a Standard Deviation of 3.8 ng/mL. The ninety-five percentile normal high cut-off is 17 ng/mL based on this study group.

A validation study of pre- and post-menopausal woman, as well as a group of male subjects, indicated a well differentiation of serum osteocalcin level of post menopausal woman from other two groups with this ELISA. The data is summarized in the following table and figure.

	Premenopausal Woman (n = 16)	Postmenopausal Woman (n = 19)	Male (n = 15)
Age			
Mean	29.0	68.7	50.3
SD	6.3	7.9	9.9
Range	21 – 40	60 – 91	37 – 76
Osteocalcin (1-43/49), ng/mL			
Mean	10.3	13.8	10.8
SD	3.0	5.0	3.6
Range	5.4 – 15.2	3.9 – 21.6	5.4 – 15.1



Forty serum samples from patients with end stage renal diseases on hemodialysis were also measured with this ELISA. Except one patient, all other 39 patients showed their osteocalcin values above the normal high cut-off ranging from 21 ng/mL to 119 ng/mL with a mean value of 60.6 ng/mL
(median: 59.6 ng/mL, SD: 26.2 ng/mL).

LIMITATION OF THE PROCEDURE

1. An abnormally high osteocalcin value is likely to indicate a more significant bone turnover condition of a patient. For sample values reading greater than highest standard, it is recommend to re-assay sample with dilution.
2. Different age group and gender may show a different normal range of osteocalcin.
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

15. PERFORMANCE

Sensitivity	The sensitivity of this human osteocalcin ELISA as determined by the 95% confidence limit on 8 replicate determinations of both zero and level 2 standards is approximately 0.31 ng/mL			
High Dose "hook" effect	This assay has showed that it did not have any high dose "hook" for sample osteocalcin level up to 1.250 ng/mL.			
Precision	Concentration (ng/mL)	CV (%)	n	
Intra-Assay	11.9 40.2	4.7 5.5	16 16	
Inter-Assay	5.6 11.9	8.3 5.7	6 6	
Linearity	Dilution	Observed Value (ng/mL)	Expected Value (ng/mL)	Recovery (%)
	Neat 1:2 1:4	69.6 34.5 15.1	- 34.8 17.4	- 99 87
	Neat 1:2 1:4	42.1 21.4 10.4	- 21.1 10.5	- 101 99
Recovery	Concentration (ng/mL)	Spiked Value (ng/mL)	Observed Value (ng/mL)	Recovery (%)
	33.4 33.4 33.4	8 16 32	18.5 23.8 30.4	89 96 93
	15.7 15.7 15.7	8 16 32	11.4 15.3 24.4	96 96 102

16. PRODUCT LITERATURE REFERENCES

1. Rosenquist C, Qvist P, Bjarnason N, Christiansen C. Measurement of a more stable region of osteocalcin in serum by ELISA with two monoclonal antibodies. Clin Chem. 1995 Oct;41(10):1439-45.

2. Takahashi M, Kushida K, Nagano A, Inoue T. Comparison of the analytical and clinical performance characteristics of an N-MID versus an intact osteocalcin immunoradiometric assay.

Clin Chim Acta. 2000 Apr;294(1-2):67-76.

3. Nagasue K, Inaba M, Okuno S, Kitatani K, Imanishi Y, Ishimura E, Miki T, Kim M, Nishizawa Y. Serum N-terminal midfragment vs. intact osteocalcin immunoradiometric assay as markers for bone turnover and bone loss in hemodialysis patients. Biomed Pharmacother. 2003 Mar;57(2):98-104.

4. Garnero P, Grimaux M, Seguin P, Delmas PD. Characterization of immunoreactive forms of human osteocalcin generated in vivo and in vitro. J Bone Miner Res. 1994 Feb;9(2):255-64

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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