EPITOPE DIAGNOSTICS, INC.

EDI™ Human Osteocalcin (1-43/49) Specific CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of Human Osteocalcin (1-43/49)inSerum or Plasma.

REF CL0809 CE IVD 100, 150,250

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human osteocalcin levels in serum or plasma using the ECL100 or ECL-25 Immunoassay analyzer. It is for in-vitro diagnostics use only.

SUMMARY OF PHYSIOLOGY

Osteocalcin is a bone gamma-carboxyglutamic acid-containing protein (BGLAP) hormone found in bone and dentin secreted by osteoblasts with synthesis involving vitamins k and D3. Intact Osteocalcin (1-49) and its fragments including N-terminal fragment osteocalcin (1-43) are then released in the bloodstream[1,4]. It plays a role in metabolic regulation[7] with its carboxylated form binding to calcium[1]and uncarboxylated form acting as a signaling hormone in the pancreas, fat, muscle, testes, and brain[6]. Clinical applications may include bone turnover[8], osteoporosis[3], hyperparathyroidism[5], and bone metasisis[2].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of human osteocalcin level in serum samples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of osteocalcin.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, anacridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles – biotin osteocalcin antibody –osteocalcin – acridinium ester osteocalcin antibody”.

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL-25 analyzer. The relative light units (RLU) are proportional to the concentration of osteocalcin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum osteocalcin concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

1. Osteocalcin Magnetic Particle Solution (L0301)

Qty:	1 x 2.8 mL (100/kit), 2 x 2.5 mL (150/kit), 3 x 2.5 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

2. Biotin Osteocalcin Antibody (L0302)

Qty:	1 x 8.5 mL (100/kit), 1 x 13 mL (150/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

3. Acridinium Ester OsteocalcinAntibody (L0303)

Qty:	1 x 8.5 mL (100/kit), 1 x 13 mL (150/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

4. Human Osteocalcin Calibrators Levels 1 and 2 (L0304, L0308)

Human osteocalcin in a lyophilized bovine serum-based matrix with a non-azide, non-mercury preservative. Refer to each vial for exact concentration.

Qty:	2 x Vials
Storage:	2 – 8°C (Lyophilized), <-20°C(Reconstituted) Do not exceed 6 freeze-thaw cycles.
Preparation:	Must be reconstituted with 0.5 mL of demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

5.Human Osteocalcin Controls (L0309, L0310)

Human osteocalcin in a lyophilized bovine serum- based matrix with a non-azide, non-mercury preservative. Refer to each vial for exact concentration.

Qty:	2 x Vials
Storage:	2 – 8°C (Lyophilized),<-20°C (Reconstituted) Do not exceed 6 freeze-thawcycles.
Preparation:	Must be reconstituted with 0.5 mLof demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. Immunoassay Analyzer (ECL100) or (ECL-25)
2. ECL100 Cuvettes (CL011) or (CL010)
3. EDI™ Wash Reagent (P-594)
4. EDI™ Trigger SolutionsA and B (P-595A, P-595B)

SPECIMEN COLLECTION AND PREPARATION

Only 50 µL of human serum or plasma sample is required for human osteocalcin measurement in duplicate. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours 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. Serum samples should be stored at 15-25°C for three days, 2-8°C for five days, and –20°C or below for three months. Avoid more than three freeze-thaw cycles of specimen. It is necessary to take care in the sample collection procedure to avoid hemolysis.

Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:

- bilirubin 60 mg/dL
- triglycerides 1500 mg/dL
- hemoglobin 900 mg/dL
- biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after takingthe last dose of biotin

A single assay of this item requires 25 µL sample.This quantity does not include the amount of dead volume in the sample container, the capacity required for retesting, and other measurement items.For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges and insert soft caps.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL-25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
Osteocalcin Controls (L0309-L0310)	25	-
Samples	-	25
Biotin Osteocalcin Antibody (L0302)	75	75
Acridinium Ester Osteocalcin Antibody (L0303)	75	75
Osteocalcin Magnetic Particle Solution (L0301)	25	25
Incubate Period		
Wash the reaction cup 3 times with the wash solution.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The first result is less than 25 minutes after loading the test

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers.Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Osteocalcin concentrations were measured in serum samples collected from 1,175 apparently healthy adults using the EDI™ Human Osteocalcin (1-43/49) Specific CLIA Kit. The observed range of osteocalcin is summarized in the table below.

	n	Osteocalcin Concentration (ng/mL)
Female		
18 – 39 Years Old	350	6.87 – 19.89
31 – 45 Years Before Menopause	249	6.55 – 18.96
≥ 50 Years Old and Post Menopause	124	7.79 – 23.98
Male		
19 – 39 Years Old	353	8.90 –25.20
31 – 45 Years Old	213	8.20 – 20.19

≥ 50 Years Old	142	6.97 – 22.59

It is highly recommended that each laboratory should establish their own normal range for osteocalcin based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL-25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 2,000 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates of blank samples to be 1.1 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 2.1 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 3.2 ng/mL.

Linearity

Linearity was determined by three replicates of each of the standards used to generate the multi-point calibration curve.

Standard	Average Concentration (ng/mL)	Theoretical Concentration (ng/mL)	CV (%)	R^2
1	0.0	0.0	0	0.990
2	15.9	13.6	3.6	
3	23.8	27.2	2.5	
4	41.8	40.8	2.1	
5	52.8	54.4	1.4	
6	71.6	68.0	2.3	

Repeatability

Reproducibility was determined by measuring ten replicates of controls.

Standard	Average Concentration (ng/mL)	CV (%)
1	9.1	6.0
2	17.7	3.0

Accuracy

Accuracy was determined by three replicates of one of the middle standards used to generate the multi-point calibration curve.

Standard	Average Concentration (ng/mL)	Target Value ± 15% (ng/mL)
3	31.85	29.8 – 40.3

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	CE European Conformity	LOT Lot Number
REF Catalog Number	 Read instructions before use	 Number of Tests
 Store at	 Use by	 Keep away from heat and direct sun light
 Manufacturer	EC REP Authorized Representative in Europe	