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Datasheet for ABIN1305168

Osteocalcin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Osteocalcin (BGLAP)
Binding Specificity:	AA 1-43, AA 1-49
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.30-65 ng/mL
Minimum Detection Limit:	0.3 ng/mL
Application:	ELISA

Product Details

Purpose:	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.
Brand:	ED™
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	1. Streptavidin Coated Microplate

Product Details

Two vials each contain 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.

Both controls should be stored at 2-8 °C and are stable until the expiration date on the kit box.

Material not included:

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 L
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.

Target Details

Target: Osteocalcin (BGLAP)

Alternative Name: Osteocalcin ([BGLAP Products](#))

Target Type: Antibody

Background: 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 bloodstream 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) is also generated by catabolic breakdown of osteocalcin (1-49) in the circulation, liver and kidney, as well as by in vitro degradation during storage of samples, because of 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

Target Details

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.

Application Details

Sample Volume: 50 µL

Assay Time: 4 h

Plate: Pre-coated

Protocol: 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

Reagent Preparation: (1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) Reconstitute all assay standards and controls by adding 0.5 mL of distilled or demineralized water to each vial. Allow the standards and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls must be stored at - 18 C or below. Do not exceed 3 freeze-thaw cycles.

Sample Collection: 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. Serum sample is allowed to be stored at 2-8 °C or room temperature for 6 days until measurement. Sample should be stored in frozen condition (< -20

°C) for longer storage. Avoid more than three freeze-thaw cycles of specimen. It is necessary to take care in the sample collection procedure to avoid haemolysis.

Assay Procedure:

- (1) Place a sufficient number of streptavidin-coated microwell strips (Cat. 10040B) in a holder to run human osteocalcin standards, controls and unknown samples in duplicate. The unused strips should be resealed in the bag with a desiccant and stored at 2-8 °C.
- (2) Test Configuration
- (3) Prepare working HRP conjugated Osteocalcin Antibody and Biotinylated Osteocalcin Antibody by 1:21 fold dilution of the conjugation antibody with the biotinylated antibody solution. Following is a table that outlines the relationship of strips used and antibody mix prepared.
- (4) Add 25 µL of standards, controls and patient serum/plasma samples into the designated microwell.
- (5) Add 200 µL of above antibody mixture to each well
- (6) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
- (7) Incubate the plate at room temperature, shaking 350 rpm 100 rpm for 1 hour.
- (8) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL - 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (9) Add 200 µL of ELISA HRP Substrate into each of the wells.
- (10) Cover the plate with one new plate sealer and also with aluminum foil to avoid exposure to light.
- (11) Incubate plate at room temperature static for 20 minutes. (This incubation period may be reduced to 8 ? 15 min if a lower OD reading is demanded to fit to the plate readers specification.)
- (12) Remove the aluminum foil and plate sealer. Add 50 µL of ELISA Stop Solution into each of the wells. Mix gently. (13) Read the absorbance at 450 nm within 10 minutes in a microplate reader. NOTE: in case extremely low background is required, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm, 620 nm or 630 nm.

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

Application Details

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.

Assay Precision: The intra-assay precision is validated by measuring two patient samples in a single assay with 16 replicate determinations. The inter-assay precision is validated by measuring two control samples in duplicate in 6 individual assays.

Restrictions: For Research Use only

Handling

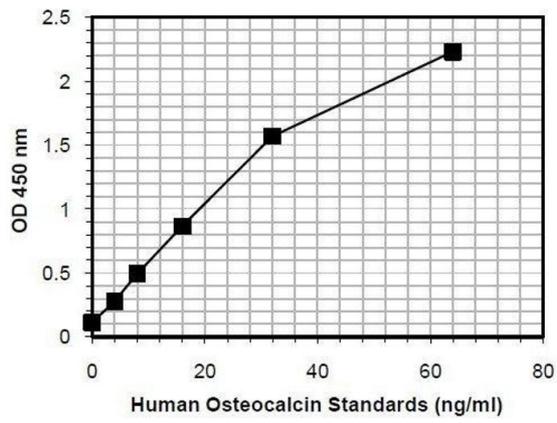
Precaution of Use: The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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.

Storage: 4 °C

Publications

Product cited in: Kamiie, Ohtsuki, Iwase, Ohmine, Katsukura, Yanai, Sekine, Uchida, Ito, Terasaki: "Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria." in: **Pharmaceutical research**, Vol. 25, Issue 6, pp. 1469-83, (2008) ([PubMed](#)).

Human Osteocalcin (1-43/49) ELISA



ELISA

Image 1.