Biomedical Technologies Inc.

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MID-TACT HUMAN OSTEOCALCIN EIA KIT
Catalog Number: BT- 480

96 Well Tests Storage 4◦C

For the measurement of Human Osteocalcin in Serum or Heparinized Plasma.

Introduction

Osteocalcin, the vitamin K-dependent protein of bone, is a specific product of the osteoblast. It is distinguished by its small size (5800 daltons) and the content of gamma-carboxy-glutamic acid (Gla). In the presence of ionic calcium, the Gla residues allow a specific conformational change in the protein which in turn promotes osteocalcin binding to bone mineral and subsequent accumulation in bone matrix. While osteocalcin is primarily deposited into the extracellular matrix of bone, a small amount can be detected in the blood. Circulating osteocalcin is thought to reflect that portion of newly synthesized protein that does not bind to bone but is released directly into the circulation.

Recent studies have shown that osteocalcin may circulate both as the intact molecule and as major N-terminal fragment (1-43). In addition, long-term storage, multiple freeze-thaw cycles and sample handling degrades osteocalcin via serum proteases. The BTI Mid-Tact Human Osteocalcin EIA Kit measures these molecular species inclusively, and will provide a more accurate assessment of osteocalcin synthesis and, therefore, bone formation.

Principle of the Assay

This is a sandwich assay utilizing two antibodies. An antibody prepared against the 1-19 fragment is immobilized on the wells of a 96-well microtest plate. Samples and a biotinylated mono-specific polyclonal antibody made against the 30-40 region are incubated in the test wells. After a wash, a second incubation is done with a Streptavidin-Horseradish Peroxidase conjugate and the enzyme activity subsequently determined. The concentration of osteocalcin in the sample is proportional to the absorbance and values are obtained by comparison to a standard curve prepared on the same plate.

Reference

1. Garnero P., Grimaux M, Seguin P. and P.D. Delmas. Characterization of Immunoreactive Forms of Human Osteocalcin in vivo and in vitro. J. of Bone & Mineral Res. 9(2): 255-264, 1994.
2. Calvo, M.S.,Eyre,D.R. and Gundberg,C.M. Molecular Basis and Clinical Application of Biological Markers of Bone Turnover. Endocrine Reviews 17(4):333-368, 1996

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR DIAGNOSTIC PROCEDURES (REV.04/03)

Reagents: Description and Preparation

All reagents Stable at 4◦C for 6 months.

1. Diluent Buffer. **BT-461**. One 30 ml bottle. Store at 4◦C. Stable for 6 months.

2. Phosphate-Saline buffer concentrate (Wash buffer). **BT-492** One 100ml bottle. Dilute contents to 500 ml with deionized water. Store at 4◦C. Stable for 6 months.

3. Osteocalcin Standards. **BT-463**. Five vials, Lyophilized. Reconstitute each vial with 0.5ml deionized water,(use 0.50ml volumetric pipet), replace stoppers and let stand for 5 minutes. Mix each vial end over end several times to obtain a clear solution. Store these reconstituted standards frozen at -20◦C (Stable for 6 months). Thaw completely and allow reconstituted standards to reach room temperature prior to use. Stable for 2 freeze thaw cycles

4. Osteocalcin Antiserum. **BT-484**. One Vial, 11ml. Biotinylated antibody to human osteocalcin. Store at 4◦C. Stable for 6 months.

5. Streptavidin Horseradish Peroxidase. **BT-465**. One Vial, 11ml.
   Store at 4◦C. Stable for 6 months.

6. Peroxidase Substrate. TMB (3,3',5,5' Tetramethylbenzidine). **BT-497**. One Vial.
   Store at 4◦C. Stable for 6 months.

7. Stop Solution (Sulfuric Acid). **BT-499**. One Vial. Store at 4◦C.
   Stable for 6 months.

8. Hydrogen Peroxide Solution. **BT-498.** One Vial. Store at 4◦C.
   Stable for 6 months.

9. Human Osteocalcin Controls. Two Vials. Add 200ul deionized water to each, let stand 10 minutes at room temperature, gently mix by inversion. (**BT-469H**, High control 25ng/ml) and (**BT-469L**, Low control 5ng/ml).

10. One 96-Well (8 strip removable well) plate, coated with 1-19 monoclonal antibody.

Other Supplies Required

1. ELISA Plate Reader which can measure absorbance at 450nm.
2. Pipettes: 100ul and 20ul micropipettes. A 0.5ml volumetric pipet.
3. A plate washer is recommended for washing.
4. A 37◦C Incubator.
5. Deionized water.

Precautions

Some components of this kit contain isothiazolones (5ppm) as preservative. Stop solution contains sulfuric acid. Keep these materials away from the skin and eyes.

Sample Collection and Storage

All samples (serum, plasma, cell culture media, etc.) should be aliquoted and stored at -20◦C. For long term storage (6-12 months) store at -70◦C. All samples should undergo only one or two freeze- thaw cycles. Serum or heparinized plasma are ideal for blood samples. Use the diluent buffer (**BT-461**) for sample dilutions.

Assay Procedure

All reagents should be at room temperature.

1. Please refer to page 2 for preparation of reagents. All reagents **must be at room temperature.**

2. Remove microtiter plate from resealable bag. Strips not used immediately should be removed from the frame and resealed in the bag for future use.

3. Add 25ul diluent buffer (zero or blank), standards, samples and controls to appropriate wells followed by 100ul osteocalcin antiserum. The entire plate should be completed in 15 minutes or less. Gently swirl the plate about 1 minute. Cover tightly and incubate at 37◦C, 2 ½ hours.

4. Aspirate completely and wash the plate 3 times with 0.3ml phosphate-saline wash buffer.

5. Add 100ul Streptavidin-Horseradish Peroxidase reagent to all wells. Swirl and then incubate at room temperature for 30 minutes.

6. Mix one volume of TMB solution (**BT-497**) with one volume of Hydrogen Peroxide solution (**BT-498**) and put aside (only mix an amount sufficient for the number of wells in use). Wash plate as in step 3. Immediately add 100ul of substrate mix to all wells, incubate at room temperature, in the dark, 15 minutes.

7. Add 100ul stop solution to all wells, swirl, and measure absorbance immediately at 450nm. Collect data.

Notes

1. Add stop solution in the same order to the plate as the substrate.

2. Before absorbance measurements are taken, be sure there are no air bubbles floating on top and the bottom of the wells are clean and dry.

3. Avoid crosscontamination by using new pipet tips for each standard and sample. Dispensing samples and standards at bottom of the wells and reagents near the top. Do not agitate or strike the plate so briskly as to cause droplets of liquid to fly up from the wells.

Calculation of Results

Average duplicates for all determinations. Subtract the zero (blank) standard from all averaged readings. Plot optical density of the standards vs. log of the concentration of each, draw the best curve. Obtain concentration of each unknown from this standard curve. Always generate a standard curve for each new assay.

Specifications

Sample size: 25ul
Assay time: 3 ½ hours
Sensitivity: 0.5 ng/ml
Working Range: 1.0-50ng/ml (450nm)
Intraassay variation: 7.0% (95% limits)
Interassay variation: 10.0% (95% limits)
Reference Interval for normal adult males and premenopausal females: 2.5-14ng/ml
High Dose "Hook" at >250ng/ml.

Typical Data

Do not use for determination of unknowns.

Standard (ng/ml)	A_{450nm}	Avg-blank
0.00 (Blank)	0.169	
0.00	0.172	(0.170)
1.00	0.255	
1.00	0.281	0.098
5.00	0.735	
5.00	0.683	0.539
10.00	1.138	
10.00	1.068	0.933
25.00	1.695	
25.00	1.620	1.488
50.00	2.087	
50.00	1.995	1.870

Typical Standard Curve