

Biomedical Technologies Inc.

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INTACT HUMAN OSTEOCALCIN EIA KIT

Catalog No: BT- 460

96 Well Tests Storage 4°C

For the Measurement of Human, Monkey, Dog and Bovine 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 presence 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.

Several recent studies suggest that there are various forms of osteocalcin in the circulation and that different antibodies detect different subforms or fragments of osteocalcin. Many polyclonal antibodies detect both intact and fragmented osteocalcin. The physiological significance of such osteocalcin fragments is unclear but they may be derived from osteoclastic resorption of matrix, osteoblastic synthesis, systemic catabolism, or all of these.

Principal of the Assay

The assay measures only intact osteocalcin, which is synthesized de novo by the osteoblast, and it eliminates any potential confounding interference by circulating fragments. The assay is a sandwich EIA which utilizes monoclonal antibodies directed toward the amino- and carboxy- terminal regions of the protein. It recognizes only intact osteocalcin, requiring the full 49 residue protein for detection. It is rapid, sensitive and reliable.

References

1. Gundberg, C.M. and R.S. Weinstein. Multiple immunoreactive forms of osteocalcin in uremic serum. *J.Clin.Invest.* 77: 1762-67, 1986.
2. Power, M.J.; Gosling, J.P. and P.F. Fotrell. Radioimmunoassay of osteocalcin with polyclonal and monoclonal antibodies. *Clin.Chem.* 35: 1408-15, 1989.
3. Tracy, R.P.; Andrianorivo, A.; Riggs, B.L. and K.G. Mann. Comparison of monoclonal and polyclonal antibody-based immunoassays for osteocalcin: a study of sources of variation in assay results. *JBMR* 5: 451-61, 1990.
4. Garner, P.; Grimaux, M.; Demiaux, B.; Preaudat, C., Seguin, P. and P.D. Delmas. Measurement of serum osteocalcin with a human-specific two-site immunoradiometric assay. *JBMR* 7: 1389-1398, 1992.
5. 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.
6. Bodine, P.V.N., Trailsmith, M., and Komm, B.S., Development and Characterization of a Conditionally Transformed Adult Human Osteoblastic Cell Line. *JBMR*, 11(6): 806-819, 1996.
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FOR RESEARCH USE ONLY

(REV. DATE: 08/09)

Reagents: Description and Preparation

All reagents stable at 4°C for 6 months.

1. Sample Buffer. **BT-471**. One 60 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 Standard. **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. Biotinylated Antiserum. **BT-464**. One Vial, 0.25ml. Biotinylated antibody to human osteocalcin. Dilute with sample buffer sufficient antiserum for current use and according to dilution ratio printed on the label.
5. Native Human Osteocalcin Antiserum. **BT-466**. One Vial, 0.5ml. Dilute contents with 10ml of sample buffer and use immediately. This is a 1:20 dilution. Dilute only enough antibody for current use.
6. Streptavidin Horseradish Peroxidase. **BT-475**. One Vial, 11ml. Store at 4°C. Stable for 6 months.
7. Peroxidase Substrate. TMB (3,3',5,5' Tetramethylbenzidine). **BT-497**. One Vial. Store at 4°C. Stable for 6 months.
8. Stop Solution (1M HCl +1M H₃PO₄). **BT-499**. One Vial, 11ml. Store at 4°C. Stable for 6 months.
9. Hydrogen Peroxide Solution. **BT-498**. One Vial. Store at 4°C. Stable for 6 months.
10. 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).
11. 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 25ul micropipettes.
3. A plate washer is recommended for washing.
4. 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 (>1month) store at -70°C. All samples should undergo only one or two freeze-thaw cycles. Serum or Heparinized plasma are ideal for blood samples. Dog serum can be run neat or diluted 1:1. Use the diluent buffer (**BT-471**) for any sample dilutions. Since bovine osteocalcin (present in bovine serum) is virtually identical with human osteocalcin (and reacts in this assay) it is necessary to wash cells with/and grow in serum free media 24-48 hours prior to taking samples.

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 50ul **Native** Osteocalcin antiserum (**BT-466**)
The entire plate should be completed in 15 minutes or less. Gently swirl about 1 minute. Cover tightly and incubate at room temperature, 1 hour.
4. Add 50ul of diluted **Biotinylated** antiserum (**BT-464**) to all wells. Swirl as above, incubate at room temperature for 1 hour.
5. Aspirate completely and wash the plate 3 times with 0.3ml phosphate-saline wash buffer. 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 4. Immediately add 100ul of substrate mix to all wells, incubate at room temperature, in the dark, 10 minutes.
7. Add 100ul stop solution to all wells, swirl, 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. Dispense 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 net 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% (95% limits)

Interassay variation: 10.5% (95% limits)

Reference Interval for normal adult males and premenopausal females:2-7ng/ml.

High Dose "Hook" at >250ng/ml.

Typical Data

(Do not use for Determination of Unknowns)

<u>Standard</u> (ng/ml)	<u>A_{450nm}</u>	<u>Avg-blank</u>
0.00 (Blank)	0.149	
0.00	0.146	0.147
1.00	0.294	
1.00	0.320	0.160
5.00	0.745	
5.00	0.850	0.605
10.00	1.051	
10.00	1.031	0.894
25.00	1.242	
25.00	1.286	1.117
50.00	1.409	
50.00	1.393	1.254

Typical Standard Curve

