DRG® Osteocalcin mid-tact (human) (EIA-1733)

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Please use only the valid version of the package insert provided with the kit.

For the measurement of human Osteocalcin in serum or heparinized plasma.
FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS OR DIAGNOSTIC PROCEDURES

Article I. 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.

Article II. References / Literature

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. 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
3. Kaigler D., Krebsbach P. H., West E.R., Horger K., Huang E-C., and D.J. Mooney. Endothellial Cell Modulation of Bone Marrow Stromal Cell Osteogenic Potential. The FASEB Journal express art. 10.1096 (January 27, 2005)
4. Hotchkiss C.E., and C.P. Jerome. Evaluation of a nonhuman primate model to study circadian rhythms of calcium metabolism. Am. J. Physiol. Regul. Comp. Physiol. 275: R494-R501, 1998.

Article III. Reagents: Description and Preparation

All reagents stable at 4 °C for 6 months.

1. **Diluent Buffer**.
   One 30 mL bottle.
   Store at 4. Stable for 6 months.
2. Phosphate-Saline buffer concentrate (**Wash buffer**).
   One 100 mL bottle.
   Dilute contents to 500 mL with deionized water
   Store at 4 °C. Stable for 6 months
3. Osteocalcin **Standards**.
   Five vials, Lyophilized.
   Reconstitute each vial with 0.5 mL deionized water, (use 0.50 mL 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**.
   One Vial, 11 mL.
   Biotinylated antibody to human osteocalcin.
   Store at 4 °C. Stable for 6 months.
5. **Streptavidin Horseradish Peroxidase**.
   One Vial, 11 mL.
   Store at 4 °C. Stable for 6 months.
6. **Peroxidase Substrate**. TMB (3,3',5,5' Tetramethylbenzidine).
   One Vial.
   Store at 4 °C. Stable for 6 months.
7. **Hydrogen Peroxide Solution**.
   One Vial.
   Store at 4 °C. Stable for 6 months.
8. **Stop Solution** (1M HCl +1M H_3PO_4)
   One Vial, 1 1ml.
   Store at 4 °C. Stable for 6 months.
9. Human Osteocalcin **Controls**.
   Two Vials.
   Add 200 µL deionized water to each, let stand 10 minutes at room temperature, gently mix by inversion.
   (High control 25 ng/mL and Low control 5 ng/mL).
10. One **96-Well (8 strip removable well) plate,**
    coated with 1-19 monoclonal antibody.

Article IV. Other Supplies Required

- ELISA Plate Reader which can measure absorbance at 450nm.
- Pipettes: 100 µL and 20 µL micropipettes. A 0.5 mL volumetric pipet.
- A plate washer is recommended for washing.
- A 37 °C Incubator.
- Deionized water.

Article V. Precautions

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

Article VI. 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 is ideal for blood samples.
Use the Diluent Buffer for sample dilutions.
Since bovine osteocalcin (present in bovine serum) is virtually identical to human osteocalcin it is best to culture cells for 24-48 hours in serum free media prior to taking cell culture supernatant samples.

Article VII. Assay Procedure

All reagents should be at room temperature.

1. Please refer to chapter 4 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 **25 µL Diluent Buffer** (zero or blank), **standards**, **samples** and **controls** to appropriate wells followed by **100 µL 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 0.3 mL phosphate-saline wash buffer.
5. Add **100 µL Streptavidin-Horseradish Peroxidase** reagent to all wells.
   Swirl and then incubate at room temperature for 30 minutes.
6. **Mix** one volume of TMB Solution (reagent 6) with one volume of Hydrogen Peroxide Solution (reagent 7) and put aside (only mix an amount sufficient for the number of wells in use).
7. Wash plate as in step 4.

8. Immediately add **100 µL of substrate mix** to all wells,
   incubate at room temperature, in the dark, 15 minutes.
9. Add **100 µL stop solution** to all wells, swirl, and measure absorbance immediately at 450 nm.
   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 cross contamination 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

Article VIII. 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.

Article IX. Specifications

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

Article X. Typical Data

Do not use for determination of unknowns.

Standard (ng/mL)	A450 nm	Avarage - 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