DRG® Mouse Osteocalcin ELISA (EIA-4010)

REVISED 22 July 2010 **For Veterinary Use Only**

For the Measurement of Mouse Osteocalcin in Serum or Heparinized Plasma, cell culture supernates and bone extracts.

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.

Principle of the Assay

This sandwich ELISA Kit is specific for mouse osteocalcin only. Both carboxylated and decarboxylated mouse osteocalcin are recognized. Mouse osteocalcin can be measured directly from serum, heparinized plasma, bone extracts and cell culture supernates. A polyclonal antibody directed against the N-terminus is bound to the polystyrene wells. An overnight incubation is done with sample and biotinylated antibody specific for the C-terminus. Detection is achieved after washing and a short incubation with Peroxidase-Streptavidin Conjugate.Standards of highly purified Mouse Osteocalcin are used to generate a standard curve.

References

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Reagents: Description and Preparation

Store all reagents at 4°C up to 3 months except as noted.

CAUTION: *DO NOT USE AZIDE.*

1. Sample buffer. One 60ml bottle.

2. Wash Buffer, Phosphate-Saline Concentrate. One 100ml bottle.
   Transfer contents to a graduated cylinder, and bring volume up to 500ml with deionized water.

3. Mouse Osteocalcin Standard. One vial 50ng lyophilized.
   Reconstitute standard with exactly 1.0ml of sample buffer (yields a solution @ 50ng/ml). Use for making working standards. Store reconstituted standard at -20°C.

4. Mouse Osteocalcin Antiserum Concentrate It is now provided as a concentrate that needs to be diluted 1:20 with sample buffer.

5. Streptavidin Horseradish Peroxidase One 11ml vial.

6. Peroxidase Substrate TMB ($3,3^1,5,5^1$-tetramethyl benzidine) One 6ml vial.

7. Hydrogen Peroxide Solution . One 6ml vial.

8. Stop Solution. One 12ml vial.

9. One 96 well plate (8 well removable strips) coated with osteocalcin antibody. Extra sealing tape provided.

Other Supplies Required

1. ELISA Plate Reader which can measure absorbance at 450nm.
2. Pipettes: micropipettes 5-1000ul.
3. A plate washer is recommended for washing.
4. A Refrigerator.
5. Deionized water.

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Precautions

Some components of this kit contain isothiazolones (5ppm) as preservative. Stop solution contains hydrochloric and phosphoric acids. Keep all materials away from the skin and eyes.

Sample Preparation

Aliquot and freeze immediately all samples for future analysis. Samples containing azide cannot be assayed.

Mouse Serum

Mouse serum must be diluted at least 5 fold with sample buffer. We recommend a 1/10 to 1/20 dilution with sample buffer. Thus it is possible to quantitate osteocalcin in 5ul of mouse serum (10ul in duplicate). Serum samples can conveniently be aliquoted in 25-50ul amounts and stored at -70º C.

The design of the animal experiment is most important. Many variables effect serum osteocalcin levels: age, growth rate, hormonal status, vitamin D intake, stress, circadian rhythm, etc. It is desirable to take blood samples under the same conditions: time of day, without stress, etc. and process the serum (or plasma) the same way for each sampling. We have found a range of 40-150 ng/ml in mouse serum that we evaluated. Young mice (<60 days) can have levels of 500ng/ml or more.

Mouse Bone

Typical levels are 1.0-2.0ng osteocalcin/ug dry bone powder. EDTA extracts of bone powder can be assayed after appropriate dilution.

Mouse Osteoblast Culture Medium

Concentrations of osteocalcin in conditioned media range from <1ng/ml to 400ng/ml depending on the cell type and culture conditions. Full dilution series should be done to establish linearity of dilution. Medium and serum interference should be assessed by appropriate blanks and internal standards of medium with added mouse osteocalcin.

Fetal Bovine Serum shows little interference. Other species of sera should be tested. Culture media can generally be assayed directly.

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Assay Procedure

CAUTION: *KEEP AZIDES AWAY FROM ALL SOLUTIONS AND SAMPLES*

All Reagents must be at room temperature prior to use.

1. Please refer to page 2 for preparation of reagents and page 3 for sample preparation.

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

3. Dilute the stock standard (50ng/ml) in polypropylene tubes with sample buffer to give six standards: 1.56, 3.12, 6.25, 12.5, 25 and 50ng/ml results in a good curve.

4. Pipet 25ul of sample buffer (Blank), Standards, Controls and Unknowns into designated duplicate wells followed by 100ul of osteocalcin antiserum in each well. Cover tightly with plastic seal provided, incubate at **2-8°C, 18-24 hours**.

5. Aspirate wells completely and wash the plate with 0.3ml/well Phosphate-Saline wash buffer. Wash plate 3 times with automatic plate washer or 5 times manually. (Complete removal of wash buffer after each wash is important for good reproducibility).

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

7. Mix one volume of TMB solution with one volume of Hydrogen Peroxide solution and put aside. (Only mix an amount sufficient for the number of wells in use) Wash the plate as in step 5. Immediately add 100ul of substrate mix to all wells and incubate at **room temperature, in the dark for 15 minutes**.

8. Add 100ul of Stop Solution to all wells, swirl and measure absorbance at 450nm immediately.
   ***NOTE:** Read absorbance values **Immediately** . Exposure to light, even for a few minutes will result in increased absorbances.

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Calculation of Results

Average duplicates for all determinations. Subtract the (Blank) from all average readings. Plot net optical density of the standards vs. log of the concentration of each, and 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: Overnight
Sensitivity: 1ng/ml
Working range: 1-50ng/ml
Intraassay variation: 6% (95% limits)
Interassay variation: 8% (95% limits)

Typical Data (Do Not Use for determination of Unknowns)

ID	A_{450}	Average – Blank
0ng/ml(Blank)	.120	
0ng/ml(Blank)	.104	(.112)
1.56ng/ml	.165	
1.56ng/ml	.180	.06
3.12ng/ml	.247	
3.12ng/ml	.254	.13
6.25ng/ml	.303	
6.25ng/ml	.345	.20
12.5ng/ml	.437	
12.5ng/ml	.523	.36
25ng/ml	.667	
25ng/ml	.779	.61
50ng/ml	1.030	
50ng/ml	1.271	1.03

Typical Standard Curve