



Enabling Legendary Discovery™  
Human Uncarboxylated Osteocalcin

## ELISA MAX™ Deluxe Set

Cat. No. 446704



BioLegend's ELISA MAX™ Deluxe Set contains the components necessary for the accurate quantification of natural and recombinant Human uncarboxylated osteocalcin. The set is designed for cost-effective and accurate quantification of Human uncarboxylated osteocalcin in cell culture supernatant, serum, plasma or other biological fluids. BioLegend's ELISA MAX™ Deluxe Sets are sensitive, accurate, and robust.

**It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.**

### Materials Provided

- Human Uncarboxylated OCN ELISA MAX™ Capture Antibody (200X)
- Human Uncarboxylated OCN ELISA MAX™ Detection Antibody (200X)
- Human Uncarboxylated OCN Standard
- Avidin-HRP (1000X)
- Substrate Solution D
- Coating Buffer B (5X)
- Assay Diluent B (5X)

### Introduction

Osteocalcin is the bone's most abundant non-collagenous protein. It is produced by osteoblasts as a 49 amino acid protein with 3 glutamine acid residues (Glu 17, 21, and 24) that are gamma carboxylated in a vitamin K dependent manner. The fully carboxylated protein has a high affinity for the bone extracellular matrix. Bone resorption results in a decrease in pH, causing the decarboxylation of osteocalcin. This generates partially decarboxylated (undercarboxylated), or fully decarboxylated (uncarboxylated) osteocalcin. These forms of osteocalcin have decreased affinity for the bone extracellular matrix and are released into the bloodstream, allowing it to bind the receptor GPCR6a, acting as hormones promoting insulin sensitivity, glucose tolerance, and testosterone biosynthesis. In patients with diabetes, reduced serum levels of osteocalcin are negatively correlated with obesity and insulin resistance.

### Principle of the Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A human uncarboxylated osteocalcin specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and uncarboxylated osteocalcin binds to the immobilized capture antibody. Next, a biotinylated anti-human osteocalcin (uncarboxylated) detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of uncarboxylated osteocalcin present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

446704\_R01

### Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H<sub>2</sub>SO<sub>4</sub>
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na<sub>2</sub>HPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub>, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 μm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 μL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

### Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent B, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion return all components to appropriate storage conditions.

### Health Hazard Warnings

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details ([www.biolegend.com/support/#msds](http://www.biolegend.com/support/#msds)).
- Substrate Solution is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

### Specimen Collection and Handling

**Cell Culture Supernatant:** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

**Serum:** Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

**Plasma:** Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

### Reagent and Sample Preparation

**Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.**

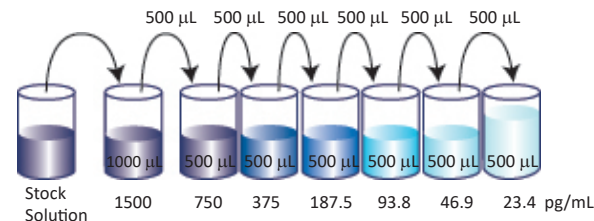
**NOTE: Precipitation of 5X Assay Diluent B may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent B, it can be filtered to clarify the solution.**

### Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 μL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL of Assay Diluent B (5X)	48 mL of PBS
60 μL of Detection Antibody (200X)	12 mL of 1X Assay Diluent B
12 μL of Avidin-HRP (1,000X)	12 mL of 1X Assay Diluent B

Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard with 1 mL of 1X Assay Diluent B. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1,000 μL of the top standard at a concentration of 1500 pg/mL from the stock solution in 1X Assay Diluent B (refer to **Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol**). Perform six two-fold serial dilutions of the 1500 pg/mL top standard with 1X Assay Diluent B in separate tubes. After diluting, the human Osteocalcin standard concentrations are 1500 pg/mL, 750 pg/mL, 375 pg/mL, 187.5 pg/mL, 93.8 pg/mL, 46.9 pg/mL, and 23.4 pg/mL, respectively. 1X Assay Diluent B serves as the zero standard (0 pg/mL).



**Samples:** For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. In general, 4-fold dilution in 1X Assay Diluent B is recommended for serum and plasma samples. Samples can be diluted in 1X Assay Diluent B to fit within the range of the assay as determined by the end user.

### Assay Procedure

**Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.**

- One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer as described in Reagent Preparation. Add 100 μL of this Capture Antibody solution to all wells of a 96-well plate. Seal plate and incubate overnight (16-18 hrs) between 2°C and 8°C.
- Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay
- Wash plate 4 times with at least 300 μL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
- To block non-specific binding and reduce background, add 200 μL 1X Assay Diluent B per well.
- Seal plate and incubate at RT for 1 hour on a plate shaker 30-60 seconds at low speed (150 -250 rpm), then incubate the plate the remaining no shaking (NOTE: do not over shake plate as this will greatly reduce assay signal). All subsequent incubation with shaking should be

performed similarly.

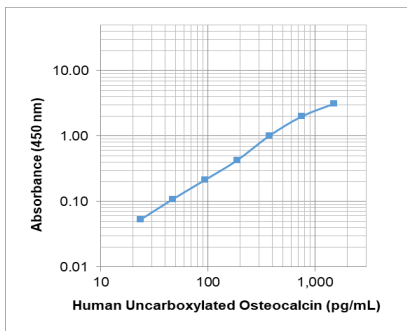
6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash plate 4 times with Wash Buffer.
8. Add 100  $\mu\text{L}$ /well of standards or samples to the appropriate wells. If dilution is required, samples should be diluted in 1X Assay Diluent B before adding to the wells.
9. Seal plate and incubate on plate shaker 30-60 seconds at low speed (150 -250 rpm), then incubate the plate overnight (23-25 hrs) at 4°C, no shaking.
10. Wash plate 4 times with Wash Buffer.
11. Add 100  $\mu\text{L}$  of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour on a plate shaker 30-60 seconds at low speed (150 -250 rpm), then the remaining time no shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100  $\mu\text{L}$  of diluted Avidin-HRP solution to each well, seal plate and incubate on plate shaker 30-60 seconds at low speed (150 -250 rpm), then the remaining time no shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100  $\mu\text{L}$  of TMB Substrate Solution D and incubate **in the dark** for 15 minutes\*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100  $\mu\text{L}$  of Stop Solution D to each well. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.  
**\*Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers**

## Calculation of Results

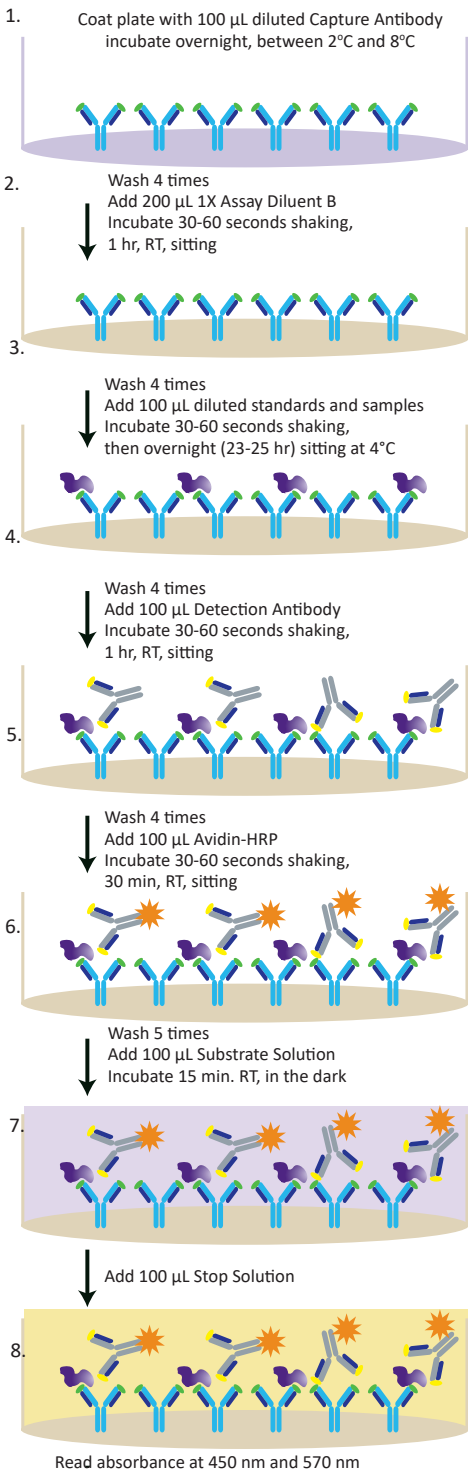
Plot the standard curve on log-log axis graph paper with analyte concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations in the samples, find the absorbance value of the unknown on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the corresponding analyte concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

## Typical Data

**Standard Curve:** This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



## Assay Procedure Summary



## Performance Characteristics

**Sensitivity:** The average lower limit of detection (LLOD) of Human Uncarboxylated Osteocalcin is 12.6 pg/mL. The average LLOD was determined using LLODs calculated from 6 independent experiments. The range of LLODs from these experiments was 11.4 - 13.4 pg/mL.

**Specificity:** No cross reactivity was observed when this kit was used to analyze multiple human recombinant proteins. Cross reactivity with fully carboxylated osteocalcin is <4.0% and undercarboxylated <5.2%.

## Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

**BioLegend, Inc.**

BioLegend is ISO 13485 certified.

8999 BioLegend Way, San Diego, CA 92121

Tel: 1-858-768-5800, Fax: 1-877-455-9587

[www.biolegend.com](http://www.biolegend.com)



For other technical resources, please visit:  
[www.biolegend.com/support](http://www.biolegend.com/support) or  
email: [techserv@biolegend.com](mailto:techserv@biolegend.com)