

HUMAN OSTEOCRIN- PREPRO ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN OSTEOCRIN-PREPRO
CONCENTRATIONS IN PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

ELISA NAME	HUMAN OSTEOCRIN-PREPRO ELISA KIT
Catalog No.	SK00012-02
Lot No.	
Formulation	96 T
Standard range	39-2500 ng/mL
Sensitivity	5 ng/mL
Sample Volume	100 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	EDTA Plasma
Specificity	Human
Calibration	Human Osteocrin-prepro Recombinant
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

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DESCRIPTION

This Human Osteocrin-prepro ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Osteocrin-prepro from EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Osteocrin-prepro and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Osteocrin-prepro samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Osteocrin-prepro. The capture antibody can bind to the human Osteocrin-prepro in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse Gas6 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of Osteocrin-prepro bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Osteocrin-prepro Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a goat polyclonal purified IgG against human Osteocrin-prepro	012-02-01	1 plate
Osteocrin-prepro Standard – 2500 ng/vial of recombinant human Osteocrin-prepro in a buffered protein base with preservative; lyophilized.	012-02-02	1 vial
Detection Antibody – 1.05 mL/vial, 10-fold concentrate of biotinylated purified IgG against human Osteocrin-prepro with preservative; lyophilized.	012-02-03	1 vial
Positive Control – one vial of recombinant human Osteocrin-prepro; lyophilized.	012-02-04	1 vial
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB18	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB06	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution – 11 mL of 0.5M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	PO1	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8 °C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8 °C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

SAMPLE COLLECTION AND STORAGE

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

EDTA plasma samples may require 2-fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

Use polypropylene test tubes.

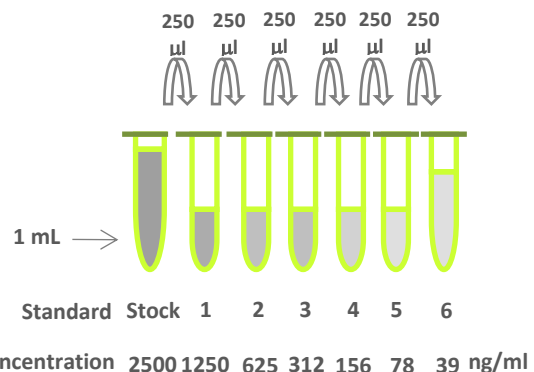
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Osteocrin-prepro Standard - Reconstitute the Human Osteocrin-prepro Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 2500 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	2500 ng/ml
# 1	250µl of stock	250µl	1250 ng/ml
# 2	250µl of 1	250µl	625 ng/ml
# 3	250µl of 2	250µl	312.5 ng/ml
# 4	250µl of 3	250µl	156.75 ng/ml
# 5	250µl of 4	250µl	78.375 ng/ml
# 6	250µl of 5	250µl	39.187 ng/ml



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Antibody Diluent Solution (DB18) to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent Solution (DB06) into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).*

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. *Note: Positive Control could be used within a few days if stored at -20 °C or -70 °C.*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

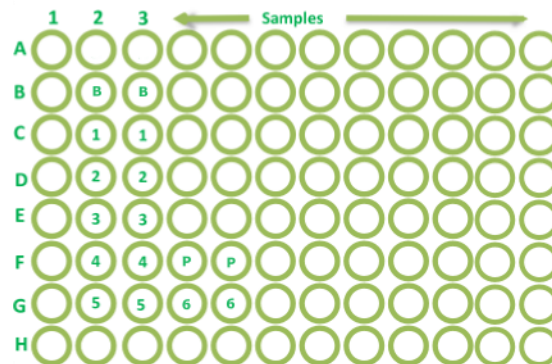
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.

7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 min on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.109)
39.18	0.065
78.375	0.117
156.75	0.227
312.5	0.414
625	0.714
1250	1.150
2500	1.721

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Osteocrin-prepro	100
Human Irisin	0
Human FNDC5	0
Human PEDF	0
Human VDBP	0
Human IL-6	0
Human MCP-1	0

LINEARITY

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2 X	322.533	322.533	100
4 X	172.207	344.414	106.8

SUMMARY OF ASSAY PROCEDURE

