Human Osteoactivin ELISA KIT

Cat. No.:DEIA7058
Pkg.Size:96T

Intended use

The Human Osteoactivin ELISA KIT is suitable for the quantitative determination of human soluble Osteoactivin/GPNMB concentrations in cell culture supernates, serum, and plasma.

General Description

Transmembrane glycoprotein NMB is a protein that in humans is encoded by the GPNMB gene. The mouse and rat orthologues of GPNMB are known as DC-HIL and Osteoactivin (OA), respectively. Two transcript variants encoding 560 and 572 amino acid isoforms have been characterized for this gene in humans. GPNMB is a type I transmembrane glycoprotein which shows homology to the pmel17 precursor, a melanocyte-specific protein.
GPNMB has been reported to be expressed in various cell types, including: melanocytes, osteoclasts, osteoblasts, dendritic cells, and it is overexpressed in various cancer types. In melanocytic cells and osteoclasts the GPNMB gene is transcritionally regulated by Microphthalmia-associated transcription factor. In osteoblast progenitor cells, Osteoactivin works as a positive regulator of osteoblast differentiation during later stages of matrix maturation and mineralization that is mediated at least in part by BMP-2 in a SMAD1 dependent manner to promote osteoblast differentiation. In addition, using a rat fracture model, Osteoactivin (OA) enhances the repairing process in bone fracture, demonstrated by its high expression during chondrogenesis (soft callus) and osteogenesis (hard callus) compared to the intact femurs that is why Osteoactivin (OA) could be a novel therapeutic agent used to treat generalized osteoporosis or localized osteopenia during fracture repair by stimulating bone growth and regeneration. Similarly, Osteoactivin expression increases during osteoclast differentiation and it is functionally implicated in this process, possibly by promoting the fusion of osteoclast progenitor cells.

Principle Of The Test

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for OSTEOACTIVIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OSTEOACTIVIN present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for OSTEOACTIVIN is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of OSTEOACTIVIN bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Osteoactivin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Osteoactivin. 1 plate
2. Osteoactivin Standard - 8000 pg/vial of recombinant human Osteoactivin in a buffered protein base with preservatives; lyophilized. 1 vial
3. Detection Antibody Concentrate - 105 µL/vial, 100-fold concentrated of biotinylated polyclonal antibody against OSTEOACTIVIN with preservatives; lyophilized. 1 vial
4. Positive Control - one vial of recombinant OSTEOACTIVIN, lyophilized. 1 vial

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5. Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugated to HRP with preservatives. 1 vial
6. Dilution Buffer - 60 mL of buffered protein based solution with preservatives. 1 bottle
7. Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative. 1 bottle
8. TMB Substrate Solution - 11 mL of TMB substrate solution. 1 bottle
9. Stop Solution - 11 mL of 0.5M HCl. 1 bottle
10. Plate Sealer. 1 piece
11. Plastic Pouch. 1 piece

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Microplate shaker (250-300rpm).
3. Pipettes and pipette tips.
4. Deionized or distilled water.
5. Squirt bottle, manifold dispenser, or automated microplate washer.
6. 100 mL and 500 mL graduated cylinders.

Storage

Unopened Kit: Store at 2 - 8 °C for up to 12 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 Antibody Solution COULD BE STORED at -20°C or -70°C for up to one month. Streptavidin- HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months. Reconstituted Positive Control should be prepared and used immediately.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

Specimen Collection And Handling

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.
SAMPLE PREPARATION
Optimal dilutions should be determined by each laboratory for each application.
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 Wash Buffer.

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OSTEOACTIVIN Standard - Refer to vial label for reconstitution volume. Reconstitute the OSTEOACTIVIN standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/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 8000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).
Table 1.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	8000 pg/ml
# 1	250µl of stock	250µl	4000 pg/ml
# 2	250µl of 1	250µl	2000 pg/ml
# 3	250µl of 2	250µl	1000 pg/ml
# 4	250µl of 3	250µl	500 pg/ml
# 5	250µl of 4	250µl	250 pg/ml
# 6	250µl of 5	250µl	125 pg/ml

Figure 1.

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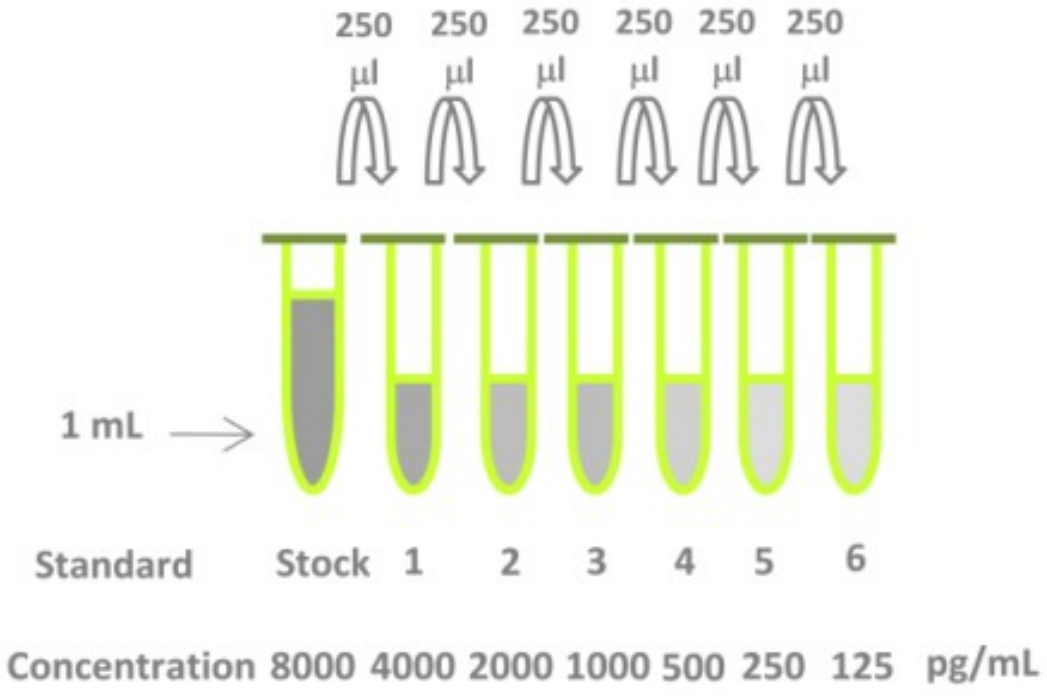

Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer. Note: Positive Control should be prepared and used immediately.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Assay Steps

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (F4, F5).
4. Add 100 µL of Standard (from B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
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 sealer. Incubate for 2 hours on micro-plate shaker at room temperature.

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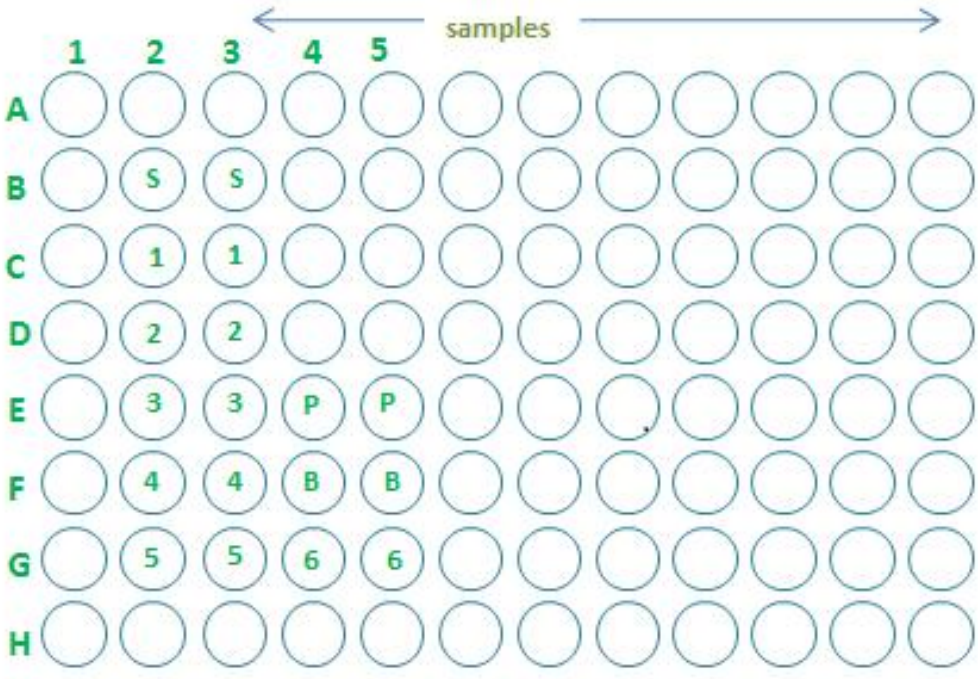
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. Protect from light.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 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 micro-plate reader set to 450 nm.
Figure 2:



Calculation

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the OSTEOACTIVIN concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.
• Positive Control: 700 - 1500 pg/ml
Table 2:

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OSTEOACTIVIN STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.109)
62.5 (optional)	0.026
125	0.045
250	0.100
500	0.208
1000	0.397
2000	0.791
4000	1.375
8000	2.407

Evaluation

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human OSTEOACTIVIN/Fc Chimera.

Detection Range

125-8000 pg/mL

Sensitivity

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of OSTEOACTIVIN was 62.5 pg/mL.

Specificity

No significant cross-reactivity or interference was observed.

Reproducibility

Intra-assay Precision: 4-6%
Inter-assay Precision: 8-10%

Limitations

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

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4. If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
5. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

Analyte Gene Information

Gene Name	GPNMB glycoprotein (transmembrane) nmb [Homo sapiens]
Official Symbol	GPNMB
Synonyms	GPNMB; glycoprotein (transmembrane) nmb; transmembrane glycoprotein NMB; glycoprotein NMB; glycoprotein nmb like protein; HGFIN; NMB; osteoactivin; transmembrane glycoprotein; glycoprotein nmb-like protein; transmembrane glycoprotein HGFIN;
GeneID	10457
mRNA Refseq	NM_001005340
Protein Refseq	NP_001005340
MIM	604368
UniProt ID	Q14956
Chromosome Location	7p
Function	heparin binding; integrin binding;

REFERENCES

1. Bozaoglu K, et al. Osteoactivin/GPNMB, a Novel Adipokine in the Regulation of Angiogenesis. J Clin Endocrinol Metab. 2010 Mar 17. [Epub ahead of print]
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