GSI Equine BMP1 ELISA Kit DataSheet

Bone morphogenetic protein 1 (BMP1), is a protein which in humans is encoded by the *BMP1* gene (1, 2). There are seven isoforms of the protein created by alternate splicing. BMP1 belongs to the peptidase M12A family of bone morphogenetic proteins (BMPs). It induces bone and cartilage development. Unlike other BMPs, it does not belong to the TGFβ superfamily. It was initially discovered to work like other BMPs by inducing bone and cartilage development. It however, is a metalloprotease that cleaves the C-terminus of procollagen I, II and III. It has an astacin-like protease domain. It has been shown to cleave laminin 5 and is localized in the basal epithelial layer of bovine skin. The BMP1 locus encodes a protein that is capable of inducing formation of cartilage in vivo. Although other bone morphogenetic proteins are members of the TGF-beta superfamily, BMP1 encodes a protein that is not closely related to other known growth factors. BMP1 protein and procollagen C proteinase (PCP), a secreted metalloprotease requiring calcium and needed for cartilage and bone formation, are identical. PCP or BMP1 protein cleaves the C-terminal propeptides of procollagen I, II, and III and its activity is increased by the procollagen C-endopeptidase enhancer protein. The BMP1 gene is expressed as alternatively spliced variants that share an N-terminal protease domain but differ in their C-terminal region.

Reference

1. Tabas JA, Zasloff M, Wasmuth JJ, Emanuel BS, Altherr MR, McPherson JD, Wozney JM, Kaplan FS (February 1991). "Bone morphogenetic protein: chromosomal localization of human genes for BMP1, BMP2A, and BMP3". *Genomics* **9** (2): 283–9
2. Mac Sweeney A, Gil-Parrado S, Vinzenz D, Bernardi A, Hein A, Bodendorf U, Erbel P, Logel C, Gerhartz B (December 2008). "Structural basis for the substrate specificity of bone morphogenetic protein 1/tolloid-like metalloproteases". *J. Mol. Biol.* **384** (1): 228–39.

PRINCIPLE OF THE ASSAY

This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance and the sensitivity of the assays. An antibody specific for equine BMP1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BMP1 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for equine BMP1 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of BMP1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

Storage

Store the standard, detection antibody at 4 °C ~ -20°C, 10 x reagent diluents and the antibody pre-coated plate at -20°C, and store the rest of the kit at 4°C. The kit can be used in 3 months.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3	Standard Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with Equine BMP1 capture antibody-**Store at -20°C upon received.

Equine BMP1 Detection Antibody– The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of 1 x Reagent Diluent to the antibody vial, vortex 20 sec and allow it to sit for 5 min. Take 200 µL of detection antibody to 9.8 mL 1 x Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Equine BMP1 Standard (3 vials) – The lyophilized Equine BMP1 Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C ~ -20 °C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. Add 500 µL of Standard Diluent to a Standard vial to make the high standard concentration of 3000 pg /ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Standard Diluent, vortex 20 sec for each of dilution step.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. If the volume is less than 50 µL, add 1 x Reagent Diluent to a final volume of 50 µL and vortex briefly. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Wash Buffer, 25 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – Store at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
Standard Diluent, 15 mL
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

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Assay Procedure

1. Lift the cover from the top left corner of the antibody coated plate and cover the wells that are not used. Vortex briefly the samples and the standards prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard to each well, cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 10 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standard at each of dilution steps is critical to ensure a normal calibration curve.
2. If BMP1 exceeds the upper limit of the detection, the sample needs to be diluted with 1 x Wash Buffer. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

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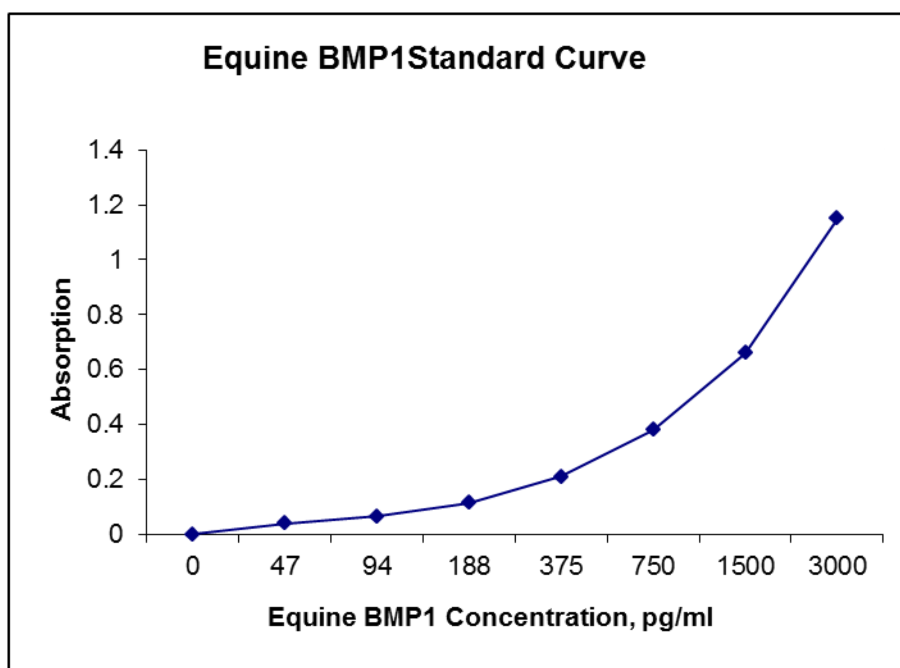
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 BMP1 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.

The Standard Curve

The graph below represents typical data generated when using this equine BMP1 ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r^2) is 0.999-1.000.



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Specificity

The following recombinant equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP2, BMP4, HGF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, VEGF.

Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant equine BMP1.

Detection Range

1-3000 pg/ml

Assay Sensitivity

0.7 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 9

For Research Use Only

Related products

20 x Sample Diluent, GERC-103058
20 x PBS, Cat. 103004-20
10 x ELISA Wash Buffer, Cat. 103028
10 x ELISA Reagent Diluent, Cat. GERC-103055
Universal Blocking Buffer, Cat.103005
2 x Recombinant Protein Stabilizer, Cat. GERC-03014-2
5 x Recombinant Protein Stabilizer, Cat. GERC-103014-5
ELISA G-Blue Substrate Solution, Cat. 103021
Equine BMP1 Standard
Equine BMP1 detection antibody