## Bovine BMP3 ELISA Kit

Bone morphogenetic protein 3 (BMP3) is a protein in humans that is encoded by the *BMP3* gene.[1] The protein encoded by this gene is a member of the transforming growth factor beta superfamily. It, like other bone morphogenetic proteins (BMP's) is known for its ability to induce bone and cartilage development. It is a disulfide-linked homodimer. It negatively regulates bone density.[2] BMP3 is an antagonist to other BMP's in the differentiation of osteogenic progenitors. BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with activin receptor type 2b.[3] It is highly expressed in fractured tissues.[4] BMP3 is downregulated in cholangiocarcinoma (CC) and may act as a biomarker of the cancer.[5] Furthermore, promotor region methylation of the BMP3 gene may cause gastric carcinoma.[6]

References

1. Faucheux C, et al. (1998). *Biochem. Biophys. Res. Commun.* **241** (3): 787–93.
2. Daluiski A, et al. (2001). *Nat. Genet.* **27** (1): 84–8.
3. Kokabu S[1], (2012) Mol Endocrinol 26:87-94.
4. Kloen P, Di Paola M, Borens O et al. (2004). *Bone* **33** (3): 362–71.
5. Kisiel JB[1], (2013) J Mol Biomark Diagn. 4(145):1000145.
6. Chen XR, et al. (2010) World J Gastroenterol. 2010 Mar 21;16(11):1409-13.

### PRINCIPLE OF THE ASSAY

This kit is for quantification of BMP3 in horse. 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 bovine BMP3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BMP3 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine BMP3 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 BMP3 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 at 4°C. The kit can be used in 3 months.

MATERIALS PROVIDED

| Description | Quantity | Description | Quantity | Description | Quantity |
|---|---|---|---|---|---|
| Antibody Precoated Plate | 1 | 20 x PBS | 1 | Substrate Solution | 1 |
| Detection Antibody | 1 | 40 x Wash Buffer | 1 | Stop Solution | 1 |
| Conjugate | 1 | 10 x Reagent Diluent | 1 | DataSheet/Manual | 1 |
| Standard | 3 | 20 x Standard/Sample Diluent | 1 | 96-well plate sheet | 1 |

Bring all reagents to room temperature before use.

**Reagent Preparations**

**Bovine BMP3 Detection Antibody** (1 vial) – The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge 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 sterile 1 x PBS to the antibody vial and vortex 20 sec. Centrifuge 1 min at 6000 x g and allow it to sit for 5 min prior to use. Take 200 µL of detection antibody to 10 mL of 1 x Reagent Diluent to make **working dilution of Detection Antibody** if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

**Bovine BMP3 Standard** (3 vials) – The lyophilized Bovine BMP3 Standard has 3 vials. Each vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°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 vial. Add 500 µL of 1 x Standard/Sample Diluent to one Standard vial to make the high standard concentration of 1,600 pg /ml. Vortex for 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 1 x Standard/Sample Diluent, each in duplicate, vortex for 20 sec for each of dilution steps.

**Conjugate** (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 50 µL Conjugate sufficient for a 96-well plate. If the volume is less than 50 µL, add sterile 1 x PBS to reach 50 µL and vortex 10 sec. Make 1:200 dilutions in 1 x Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Conjugate to 10 mL of 1 x Reagent Diluent to make **working dilution of Conjugate** prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 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**, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
**10 x Reagent Diluent**–Add 3 mL of sterile 1 x PBS to make 10 x Reagent Diluent, vortex 1 min and allow it to stay for 15 min to completely dissolve. Store at -20°C. Dilute to 1 x Reagent Diluent with PBS.
**20 x Standard/Sample Diluent**, 15 mL- Dilute to 1 x Standard Diluent with 1 x PBS prior to use.
**Substrate Solution**, 10 mL.
**Stop Solution**, 5 mL.

**Assay Procedure**

1. Lift the plate cover from the top left corner 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 and use duplicate for samples and standards, 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 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 Conjugate** 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 5-10 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 availBBle, 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. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each of dilution steps is crucial to ensure a normal standard curve.
3. If BMP3 exceeds the upper limit of the detection, the sample needs to be diluted with the 1 x Standard/Sample Diluent. The dilution factor must be used for calculation of the concentration.
4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
5. The Stop Solution is an acid solution, handle with caution.
6. This kit should not be used beyond the expiration date on the label.
7. 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.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

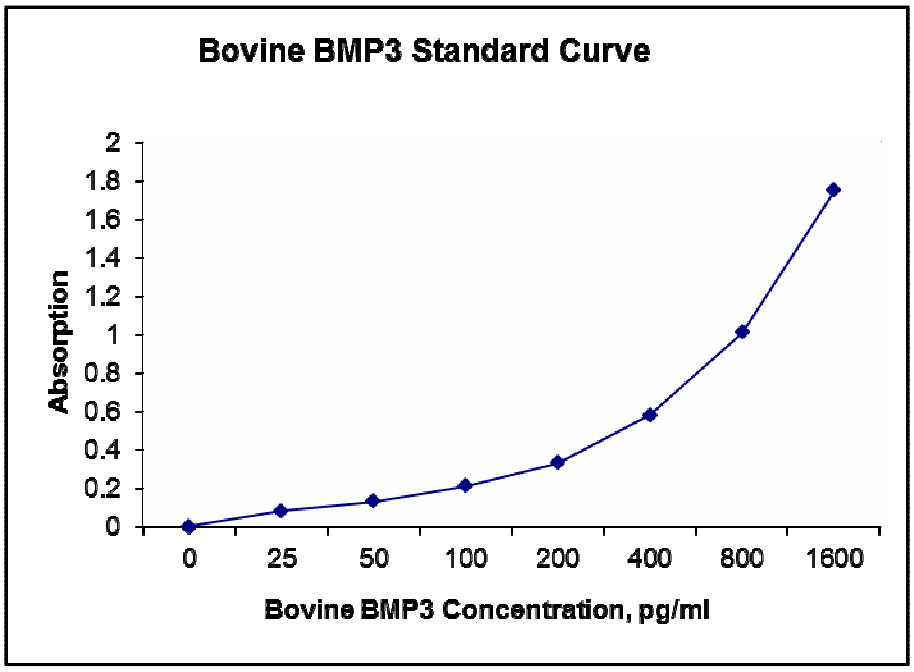
## Calculation of Results

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 BMP3 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 bovine BMP3 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.



## Specificity

The following recombinant Bovine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.

ApoAI, BMP1, BMP2, BMP3, BMP4, HGF, HSP27, IFNγ, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, IL-23, MMP-2, MMP-9, sIL-2R, sIL-6R, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF.

## Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant bovine BMP3.

## Detection Range

25-1,600 pg/ml

## Assay Sensitivity

5 pg/ml

## Assay Precision

Intra-Assay %CV: 4; Inter-Assay %CV: 9

# For Research Use Only

## Related products

1. 20 x Sample Diluent
2. 20 x PBS
3. 10 x ELISA Wash Buffer
4. 10 x ELISA Reagent Diluent
5. Universal Blocking Buffer
6. 2 x Recombinant Protein Stabilizer
7. 5 x Recombinant Protein Stabilizer
8. ELISA G-Blue Substrate Solution
9. Bovine BMP3 Standard
10. Bovine BMP3 detection antibody