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Research
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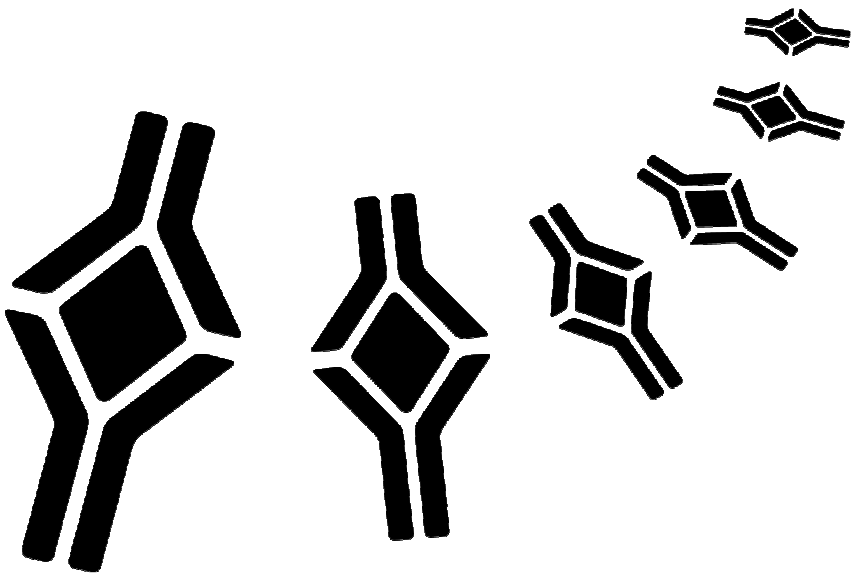


HUMAN BONE MORPHOGENETIC PROTEIN-4 (BMP-4) ELISA

Product Data Sheet

Cat. No.: BBT0314R

For Research Use Only

VERSION 51 041209

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

For quantitative detection of human BMP-4 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

2. STORAGE, EXPIRATION

Storage
Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration
Four months at 4°C and eight months at -20°C.

3. INTRODUCTION

Bone morphogenetic protein 4 is a protein that in humans is encoded by the BMP4 gene which is located to 14q22-q23.1, 2 The protein encoded by this gene is a member of the bone morphogenetic protein family which is part of the transforming growth factor-beta superfamily. BMP4 is a polypeptide belonging to the TGF-β superfamily of proteins. It, like other bone morphogenetic proteins, is involved in bone and cartilage development, specifically tooth and limb development and fracture repair. It has been shown to be involved in muscle development, bone mineralization, and uteric bud development. BMP4 has also been implicated in Fibrodysplasia Ossificans Progressiva in which it is underexpressed. In human embryonic development, BMP4 is a critical signaling molecule required for the early differentiation of the embryo and establishing of a dorsal-ventral axis. BMP4 is secreted from the dorsal portion of the notochord, and it acts in concert with sonic hedgehog (released from the ventral portion of the notochord) to establish a dorsal-ventral axis for the differentiation of later structures. BMP4 stimulates differentiation of overlying ectodermal tissue. Inhibition of the BMP4 signal (by chordin, noggin, or follistatin) causes the ectoderm to differentiate into the neural plate. The standard product used in this kit is recombinant BMP-4 with the molecular mass of 26KDa.

4. TEST PRINCIPLE

Biovendor's human BMP-4 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human BMP-4 specific-specific monoclonal antibodies (clone No. 66110) were precoated onto 96-well plates. The human specific detection monoclonal antibodies (clone No. 66108) were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human BMP-4 amount of sample captured in plate.

5. PRECAUTIONS

- Before using Kit, spin tubes and bring down all components to bottom of tube.
- Duplicate well assay was recommended for both standard and sample testing.
- Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

6. REAGENT SUPPLIED

- Lyophilized recombinant human BMP-4 standard: 10 ng/tube×2.
- One 96-well plate precoated with anti- human BMP-4 antibody.
- Sample diluent buffer: 30 ml
- Biotinylated anti- human BMP-4 antibody: 130 µl, dilution 1:100.
- Antibody diluent buffer: 12 ml.
- Avidin-Biotin-Peroxidase Complex (ABC): 130 µl, dilution 1:100.
- ABC diluent buffer: 12 ml.
- TMB color developing agent: 10 ml.
- TMB stop solution: 10 ml.

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Microplate reader in standard size.
- Automated plate washer.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- Clean tubes and Eppendorf tubes.
- Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS:** Add 1.2 g Tris, 8.5 g Nacl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

8. PREPARATION OF REAGENTS AND SAMPLES

Plate Washing

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

Serum: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma: Collect plasma using EDTA as an anticoagulant. Centrifuge for 10 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store samples at -20°C.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

High target protein concentration (40-400 ng/ml). The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

Medium target protein concentration (4-40 ng/ml). The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

Low target protein concentration (62.5-4000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

Very Low target protein concentration (≤62.5 pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the human BMP-4 standard: BMP-4 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of BMP-4 standard (10ng per tube) are included in each kit. Use one tube for each experiment.
   a. 10,000 pg/ml of human BMP-4 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
   b. 4000 pg/ml of human BMP-4 standard solution: Add 0.4 ml of the above 10 ng/ml BMP-4 standard solution into 0.6 ml sample diluent buffer and mix thoroughly.
   c. 2000 pg/ml→62.5 pg/ml of human BMP-4 standard solutions: Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 4000 pg/ml BMP-4 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human BMP-4 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
   a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Biotinylated anti-human BMP-4 antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
   a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

9. ASSAY PROCEDURE

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard BMP-4 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of BMP-4 amount in samples.

1. Aliquot 0.1ml per well of the 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml human BMP-4 standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human BMP-4 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human BMP-4 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 15-20 min (shades of blue can be seen in the wells with the four most concentrated human BMP-4 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human BMP-4 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 15-20 min.
5. Add TMB stop solution and read.

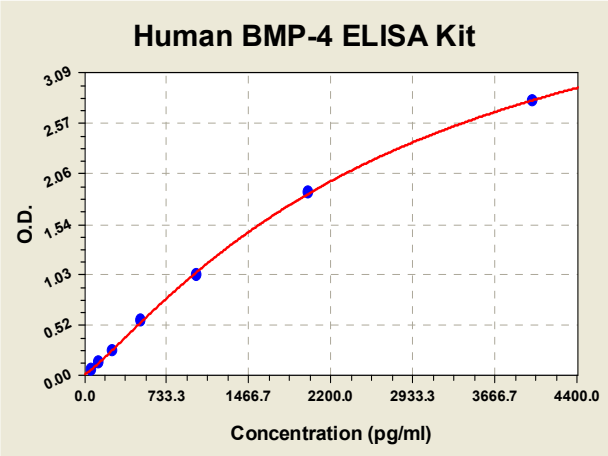
10. CALCULATIONS

(TMB reaction incubate at 37°C for 18 min)

Concentration	0.0 pg/ml	62.5 pg/ml	125 pg/ml	250 pg/ml	500 pg/ml	1000 pg/ml	2000 pg/ml	4000 pg/ml
O.D.	0.017	0.074	0.143	0.263	0.572	1.035	1.877	2.807

Typical Human BMP-4 ELISA Kit Standard Curve

This standard curve was generated at BioVendor for demonstration purpose only. A standard curve must be run with each assay.



11. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human BMP-4 ELISA are presented in this chapter.

- **Sensitivity**

< 4 pg/ml

- **Specificity**

No detectable cross-reactivity with any other cytokine.

- **Range**

62.5 pg/ml-4000 pg/ml

- **Size**

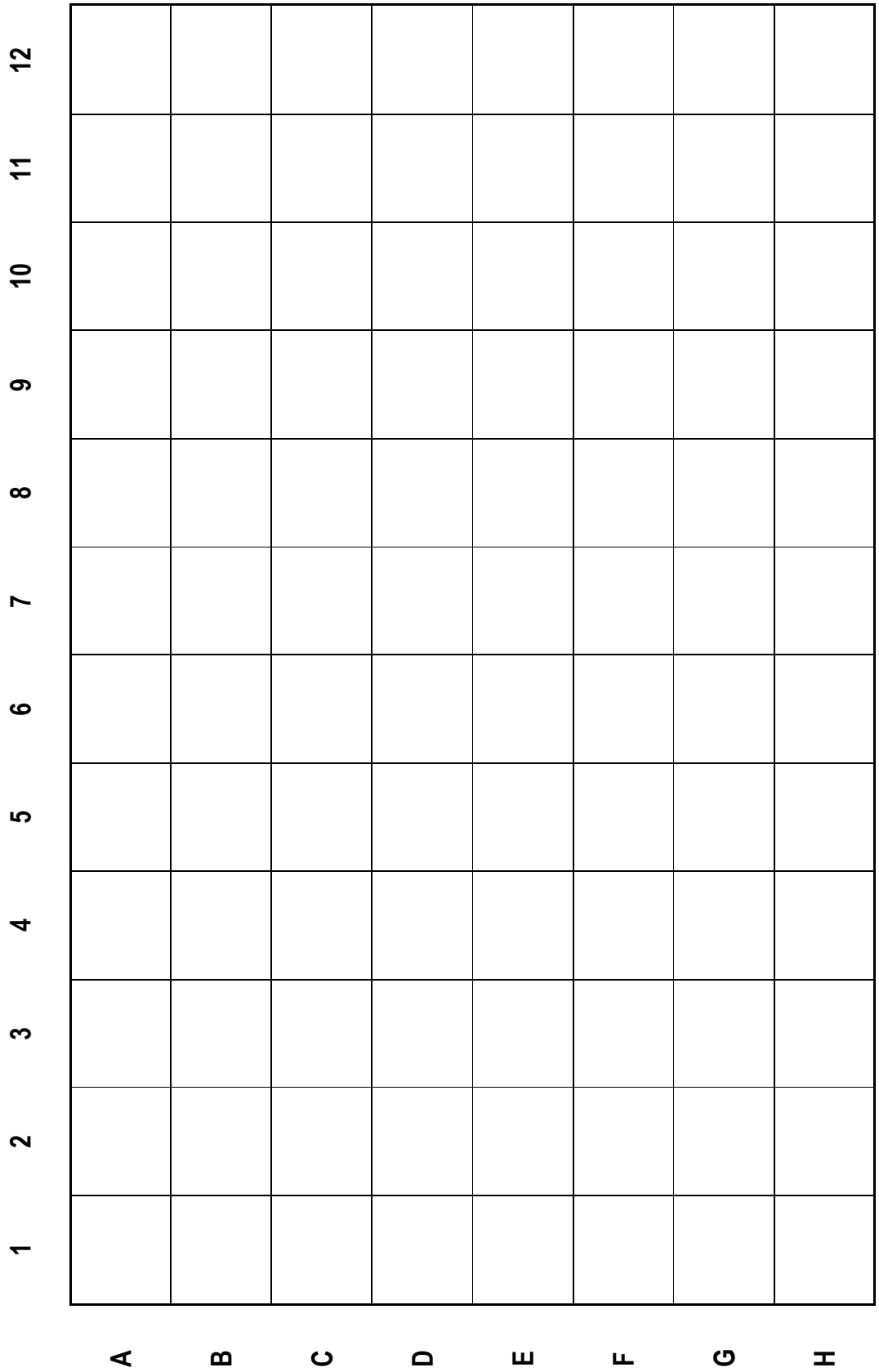
96T

12. REFERENCES

References to human BMP-4 ELISA:

1. van den Wijngaard A, Weghuis DO, Boersma CJ, van Zoelen EJ, Geurts van Kessel A, Olijve W (Nov 1995). "Fine mapping of the human bone morphogenetic protein-4 gene (BMP4) to chromosome 14q22-q23 by in situ hybridization". *Genomics* 27 (3): 559-60.
2. Oida S, Iimura T, Maruoka Y, Takeda K, Sasaki S (Nov 1995). "Cloning and sequence of bone morphogenetic protein 4 (BMP-4) from a human placental cDNA library". *DNA Seq* 5 (5): 273-5.

» For more references on this product see our WebPages at www.biovendor.com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NOTES

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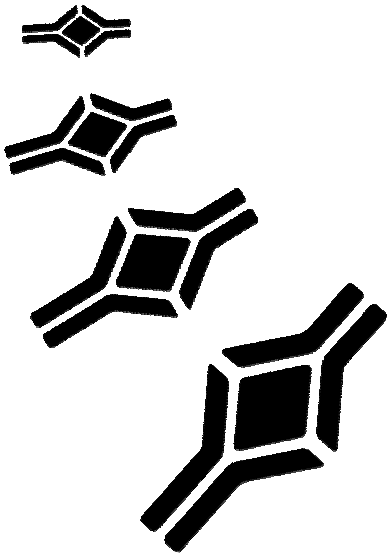

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