



## Product Information Sheet

### Human BMP-4 ELISA Kit

**Catalog No.** EK0314  
**Size** 96T  
**Range** 62.5pg/ml-4000pg/ml  
**Sensitivity** < 4pg/ml

**Specificity**

No detectable cross-reactivity with any other cytokine.

**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.

**Application**

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

**Principle**

Human BMP-4 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human BMP-4 specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal antibodies 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.

**Kit Components**

1. Lyophilized recombinant human BMP-4 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- human BMP-4 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human BMP-4 antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

**Material Required But Not Provided**

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

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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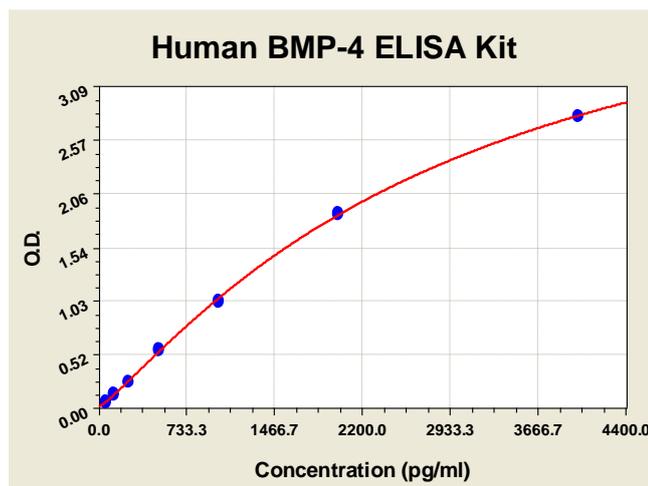
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## Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. 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.

## Human BMP-4 ELISA Kit-1X96 Well Plate Image



## Background

Bone morphogenetic protein 4 is a protein that in humans is encoded by the BMP4 gene which is located to 14q22-q23.<sup>1</sup>  
<sup>2</sup> 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- $\beta$  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 uterine 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.

## Reference

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.

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