Datasheet for ABIN624950

BMP4 ELISA Kit

1 Image | 11 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	BMP4
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	15-6000 pg/mL
Minimum Detection Limit:	15 pg/mL
Application:	ELISA

Product Details

Purpose:	Human BMP-4 ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, BMP-6, BMP-7, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GMCSF, IFN-gamma, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 15 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	BMP4
Alternative Name:	BMP4 (BMP4 Products)
Background:	BMPs (Bone morphogenetic proteins) are found in minute amounts in bone material. Some BMPs, including BMP-2, BMP-4, and BMP-7, play a role in the specification of hematopoietic tissue from the mesodermal germ layer. They regulate the proliferation and differentiation of highly purified primitive human hematopoietic cells from adult and neonatal sources. BMP-4 has been shown to be involved also in the differentiation of sympathetic neurons. It enhances the formation of adrenergic sympathetic neurons in cultures of neural crest cells. The Human BMP-4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human BMP-4 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human BMP-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and BMP-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human BMP-4 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again

Target Details

	washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of BMP-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	652
UniProt:	P12644
Pathways:	Steroid Hormone Mediated Signaling Pathway, Regulation of Muscle Cell Differentiation, Tube Formation, Skeletal Muscle Fiber Development

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernantants and urine. Suggested dilution for normal serum/plasma: 2 fold*. *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 80

	µL BMP-4 standard from the vial of Item C, into a tube with 586.7 µL Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/mL stock standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 200 µL 200myl 200 µL 200 µL 200 µL 200 µL 80 µL standard +586.7 µL 6000 2000 666.7 222.2 74.07 24.69 8.23 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 65-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 80-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 150 µL of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a final 80 fold diluted HRP-Streptavidin solution. Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette 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. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30

Application Details

	minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Human BMP-4 concentration (pg/mL) 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Human BMP-4 concentration (pg/mL) 10 100 1000 10000 O D =4 50 n m 0.1 1 10 Sensitivity: The minimum detectable dose of BMP-4 is typically less than 15 pg/mL. Recovery: Recovery was determined by spiking various levels of human BMP-4 into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 92.95 80-101 Plasma 94.32 82-102 Cell culture media 98.25 85-104 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 94 93 95 Range (%) 83-103 82-102 84-103 1:4 Average % of Expected 96 95 101 Range (%) 85-104 83-102 88-106 1:8 Average % of Expected 96 97 94 Range (%) 84-104 85-104 84-103 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Bhattacharyya, Feferman, Tobacman: "Regulation of chondroitin-4-sulfotransferase (CHST11) expression by opposing effects of arylsulfatase B on BMP4 and Wnt9A." in: **Biochimica et**

biophysica acta, Vol. 1849, Issue 3, pp. 342-52, (2015) (PubMed).

Pallotta, Sun, Wrona, Freytes: "BMP protein-mediated crosstalk between inflammatory cells and human pluripotent stem cell-derived cardiomyocytes." in: **Journal of tissue engineering and regenerative medicine**, (2015) (PubMed).

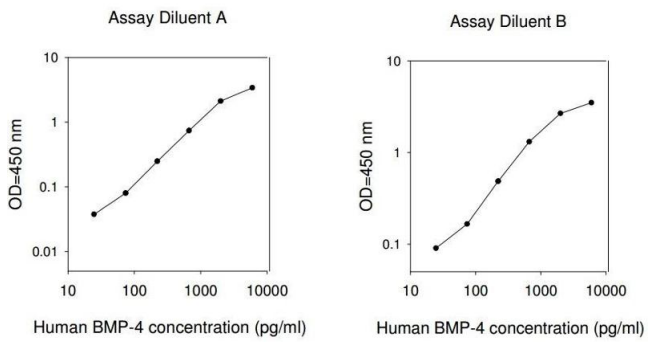
Youn, Zhou, Cai: "Bone Morphogenic Protein 4 Mediates NOX1-Dependent eNOS Uncoupling, Endothelial Dysfunction, and COX2 Induction in Type 2 Diabetes Mellitus." in: **Molecular endocrinology (Baltimore, Md.)**, Vol. 29, Issue 8, pp. 1123-33, (2015) (PubMed).

Zhang, Chen, Guo, Yuan, Zhang, Xu, Nemeth, Ganz, Liu: "Disordered hepcidin-ferroportin signaling promotes breast cancer growth." in: **Cellular signalling**, Vol. 26, Issue 11, pp. 2539-50, (2014) (PubMed).

Dincel, Sepici-Dincel: "The importance and the differences of bone morphogenetic proteins for osteoporotic hip fractures." in: **Acta orthopaedica Belgica**, Vol. 80, Issue 2, pp. 216-21, (2014) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1.