BOSTER

antibody and ELISA experts

# PicoKine® ELISA

## Catalog number: EK0314

For the quantitation of **Human BMP4** concentrations in cell culture supernatants, cell lysates and serum.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

# Human BMP-4 ELISA Kit PicoKine®

**Catalog Number:** EK0314

## Assay Principle

The Boster Picokine® Human BMP4 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human BMP4 with a 96-well strip plate that is pre-coated with antibody specific for BMP4. The detection antibody is a biotinylated antibody specific for BMP4. The kit includes Human BMP4 protein as standards.

To measure Human BMP4, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human BMP4 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human BMP4 in the sample. For more information on assay principle, protocols, and troubleshooting tips, see Boster's ELISA Resource Center at https://www.bosterbio.com/elisa-technical-resource-center.

## Submit a Product Review to Biocompare.com






Submit a review of this product to Biocompare.com to receive a $20 Amazon.com gift card! Your reviews help your fellow scientists make the right decisions. Thank you for your contribution.

## Overview

| | |
|---|---|
| Product Name | Human BMP-4 ELISA Kit PicoKine® |
| Reactive Species | Human |
| Size | 96 wells/kit, with removable strips. |
| Description | Human BMP-4 ELISA Kit PicoKine® (96 Tests). Quantitate Human BMP4 in cell culture supernatants, cell lysates and serum. Sensitivity: 4pg/ml. The brand Picokine indicates this is a premium quality ELISA kit. Each Picokine kit delivers precise quantification, high sensitivity, and excellent reproducibility. Only our most reliable and effective kits qualify as Picokine, guaranteeing top-tier results for your assays. |
| Sensitivity* | <4 pg/ml |
| Detection Range | 62.5 pg/ml - 4,000 pg/ml |
| Storage Instructions | Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.) |
| Uniprot ID | P12644 |

*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the target protein that can be detected by the kit. It is determined by

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

## Technical Details

| | |
|---|---|
| Specificity | Natural and recombinant Human BMP4 |
| Standard Protein | Expression system for standard: NS0; Immunogen sequence: S293-R408 |
| Cross-reactivity | This kit is for the detection of Human BMP4. No significant cross-reactivity or interference between BMP4 and its analogs was observed. This claim is limited by existing techniques; therefore, cross-reactivity may exist with untested analogs. |

## Preparations Before Assay

Please read the following instructions before starting the experiment.
1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

## Kit Components/Materials Provided

| Description | Quantity | Volume | Storage of opened/reconstituted material |
|---|---|---|---|
| Anti-Human BMP4 Pre-coated 96-well Strip Microplate | 1 | 12 strips of 8 wells | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Human BMP4 Standard | 2 | 10 ng/tube | Discard the BMP4 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours. |
| Human BMP4 Biotinylated Antibody (100x) | 1 | 100 µl | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 | 100 µl | |
| Sample Diluent | 1 | 30 ml | |
| Antibody Diluent | 1 | 12 ml | |
| Avidin-Biotin-Peroxidase Diluent | 1 | 12 ml | |
| Color Developing Reagent (TMB) | 1 | 10 ml | |
| Stop Solution | 1 | 10 ml | |
| Wash Buffer (25x) | 1 | 20 ml | |
| Plate Sealers | 4 | Piece | |

## Required Materials That Are Not Supplied

Microplate reader capable of reading absorbance at 450 nm.
Incubator.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for a large numbers of samples.
Deionized or distilled water.
500 ml graduated cylinders.
Test tubes for dilution.

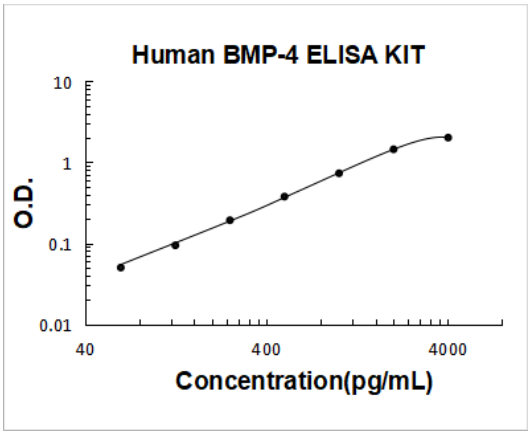
BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

# Human BMP4 ELISA Standard Curve Example

The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

| Concentration (pg/ml) | 0 | 62.5 | 125 | 250 | 500 | 1000 | 2000 | 4000 |
|---|---|---|---|---|---|---|---|---|
| O.D. | 0.015 | 0.066 | 0.111 | 0.211 | 0.399 | 0.76 | 1.489 | 2.069 |

**Human BMP-4 PicoKine ELISA Kit standard curve**




A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

# Intra/Inter-Assay Variability

Boster spends great efforts in documenting lot-to-lot variability and ensuring our assay kits produce robust data that are reproducible.

**Intra-Assay Precision (Precision within an assay):** Three samples of known concentration were tested on one plate to assess intra-assay precision.

**Inter-Assay Precision (Precision across assays):** Three samples of known concentration were tested in separate assays to assess inter-assay precision.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 16 | 16 | 16 | 24 | 24 | 24 |
| Mean (pg/ml) | 196 | 814 | 1756 | 199 | 823 | 1888 |
| Standard deviation | 12.15 | 48.02 | 124.67 | 12.33 | 49.38 | 169.92 |
| CV (%) | 6.2% | 7.1%% | 7.1% | 6.2% | 6% | 9% |

BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

## Preparation Before The Experiment

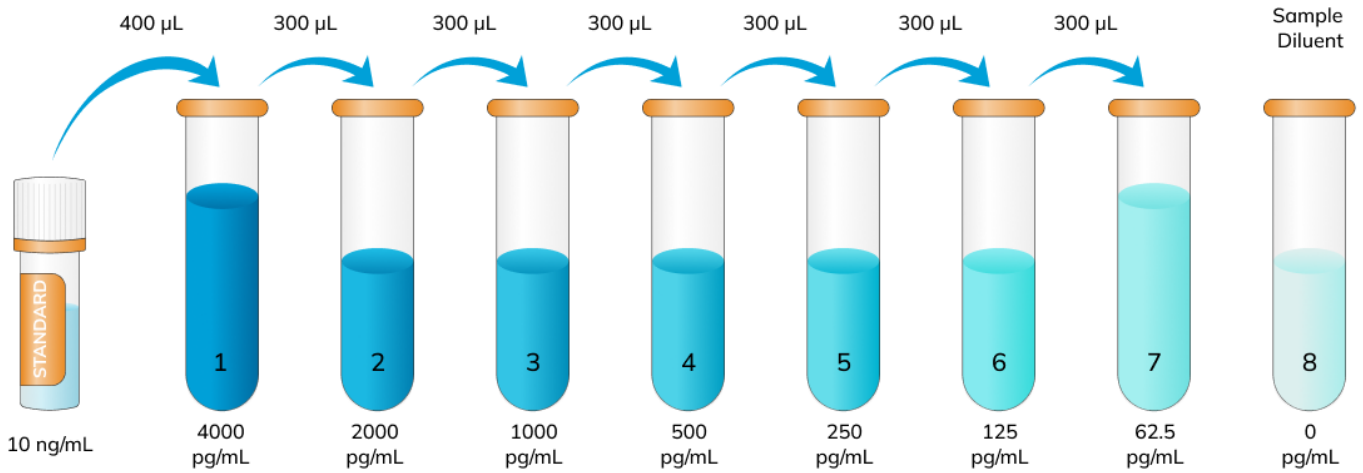
| Item | Preparation |
|---|---|
| All reagents | Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C. |
| Wash buffer | Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. |
| Biotinylated Anti-Human BMP4 antibody | It is recommended to prepare this reagent immediately prior to use by diluting the Human BMP4 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Avidin-Biotin-Peroxidase Complex | It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Human BMP4 Standard | It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human BMP4 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. |

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

| | |
|---|---|
| Microplate | The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging. |
| Samples | Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.<br>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.<br>Some PubMed article(s) citing the expression level of this target are as follows: 23208015<br>Boster Bio's internal QC testing used:<br>Dilution ratio of 1:1, concentration in serum is 0-110 pg/ml. |

## Dilution of Human BMP4 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1: 4,000.00 pg/ml, # 2: 2,000.00 pg/ml, # 3: 1,000.00 pg/ml, # 4: 500.00 pg/ml,
   # 5: 250.00 pg/ml, # 6: 125.00 pg/ml, # 7: 62.50 pg/ml, # 8: Sample Diluent serves as the zero standard (0 pg/ml).
2. To generate standard #1, add 400 µl of the reconstituted standard stock solution of 10 ng/ml and 600 µl of sample diluent to tube #1 for a
   final volume of 1000 µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard # 2, add 300 µl of standard # 1 from tube # 1 to tube # 2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard # 3, add 300 µl of standard # 2 from tube # 2 to tube # 3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube # 4-7.




## Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline, and the sample stability has not been evaluated.

Sample dilution ratios should be determined by a pilot study (run a serial dilution of samples and see which dilution ratio results in the idea O.D., near the middle of the standard range). In general, high concentration samples can be dilutioned by 1:100, mid concentration samples 1:10, low concentration samples 1:2 or neat.

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

| Sample Type | Procedure |
|---|---|
| Cell culture supernatants | Clear sample of particulates by centrifugation, assay immediately, or store samples at -20°C. |
| Serum | Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C. |
| Cell lysates | Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant. |

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

## Sample Collection Notes

1. Boster recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Boster is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

## Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

## Assay Protocol

It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the **Sample Diluent** into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared **1x Biotinylated Anti-Human BMP4 antibody** to each well.
7. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).
8. Wash the plate 3 times with the **1x wash buffer**:
 a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 b. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 c. Repeat steps a-b 2 additional times.
 d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
9. Add 100 µl of the prepared **1x Avidin-Biotin-Peroxidase Complex** into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the **1x wash buffer**:
 a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 b. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

c. Repeat steps a-b 4 additional times.
d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
11. Add 90 µl of **Color Developing Reagent** to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of **Stop Solution** to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

## Assay Protocol Notes

1. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
3. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
4. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
5. Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
6. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.
7. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
8. Reaction Time Control: Control reaction time should be strictly followed as outlined.
9. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

## Data Analysis

Boster Bio offers an easy-to-use online ELISA data analysis tool. Try it out at https://www.bosterbio.com/biology-research-tools/elisa-data-analysis-online

To analyze using manual methods, follow the process below:

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.
It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

## Background on BMP4

Bone morphogenetic protein 4 is a protein that in humans is encoded by the BMP4 gene which is located to 14q22-q23.1, 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 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.

## 2 Publications Citing This Product

1. PubMed ID: 10.1016/j.biomaterials.2021.121260, An improved osseointegration of metal implants by pitavastatin loaded multilayer films with osteogenic and angiogenic properties

2. PubMed ID: 10.1016/S0020-1383(17)30774-X, Bone intramedullary reaming grafts the fracture site with CD146+ skeletal progenitors and downmodulates the inflammatory environment

Visit bosterbio.com/human-bmp-4-picokine-trade-elisa-kit-ek0314-boster.html to see all 2 publications.



For Research Use Only. Not for use in diagnostic procedures.