Human BMP-2 ELISA Development Kit

Cat# E5041 5 plates

Store the unopened product at 2 - 8 °C.

DESCRIPTION

Human BMP-2 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant BMP-2 in a sandwich ELISA format. Using the ELISA protocol described below, this kit provides sufficient reagents to assay BMP-2 in approximately 1500 ELISA plate wells.

INTENDED USE

For the development of sandwich ELISAs to measure natural and recombinant human Bone Morphogenetic Protein 2 (BMP-2). This kit does not detect E. coli-expressed BMP-2. The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

BACKGROUND

BMPs (Bone Morphogenetic Proteins) belong to the TGF-beta superfamily of structurally related signaling proteins. BMP-2 is a potent osteoinductive cytokine, capable of inducing bone and cartilage formation in association with osteoconductive carriers such as collagen and synthetic hydroxyapatite. In addition to its osteogenic activity, BMP-2 plays an important role in cardiac morphogenesis. The functional form of BMP-2 is a 26 kDa protein composed of two identical 114 amino acid polypeptide chains linked by a single disulfide bond. Each BMP-2 monomer is expressed as the C-terminal part of a precursor polypeptide, which also contains a 23 amino acid signal sequence for secretion, and a 259 amino acid propeptide. After dimerization of this precursor, the covalent bonds between the propeptide (which is also a disulfide-linked homodimer) and the mature BMP-2 ligand are cleaved by a furin-type protease.

Human and mouse BMP-2 and BMP-7 are 100% and 98% identical, respectively, at the amino acid level. Human BMP-2 shares 85% aa sequence identity with human BMP-4 and less than 51% aa sequence identity with other BMPs. Human BMP-7 shares approximately 60% - 70% aa sequence identity with BMP-5, -6, and -8, and less than 50% aa sequence identity with other BMPs. BMP-2 and BMP-7 are co-expressed in some embryonic tissues and associate into a functional 38 kDa osteogenic dimer. In in vitro osteoblast differentiation assays and in vivo bone formation models, a BMP-2/BMP-7 heterodimer is significantly more potent than either homodimer.

Bone morphogenetic protein 2 is shown to stimulate the production of bone and recombinant human protein (rhBMP-2) and is currently available for orthopaedic usage in the United States. Implantation of BMP-2 in a collagen sponge induces new bone formation and can be used for the treatment of bony defects, delayed union, and non-union. Bone morphogenetic protein 2 has also found its way into the field of Dentistry. Oral Surgery and Implant Dentistry in

particular have benefited dramatically from commercially available BMP-2.

MATERIALS PROVIDED

1.) Capture Antibody

2.) Detection Antibody

3.) Standard

4.) StreptAvidin-HRP

5.) TMB Liquid Substrate "Ready to Use"

STORAGE CONDITIONS

Store the unopened product at 2 - 8 °C. Refer to lot-specific datasheet (C of A) for details on each component.

OTHER MATERIALS AND SOLUTIONS REQUIRED

Additional Required Materials

ELISA microplates

BSA

Dulbecco's PBS (DPBS) [10x]

Stop Solution: 450 nm Stop Reagent for TMB Microwell

Required Solutions

PBS: dilute 10xPBS to 1xPBS, pH 7.2, in sterile water

Wash Buffer: 0.05% Tween-20 in PBS

Reagent Diluent: 1.0% BSA in PBS

Blocking Buffer 1.0% BSA in PBS

PRECAUTIONS

Some of the required components may contain acid and/or cause allergic reactions. Breathing in product mist or fumes should be avoided. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

CAPTURE ANTIBODY

Mouse Anti-Human BMP-2 capture antibody: Centrifuge vial prior to opening. Reconstitute in 0.5 mL sterile PBS. Refer to the lot-specific datasheet for amount supplied. Following reconstitution the Capture antibodies may be stored at 2 – 8°C for up to 6 months. For long term storage, it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer. **Avoid repeated freeze and thaw cycles.**

BIOTIN DETECTION ANTIBODY

Biotinylated Mouse Anti-Human BMP-2: Refer to the lot-specific datasheet for amount supplied. Centrifuge vial prior to opening. Reconstitute with 1.0 mL of reagent diluent. Dilute in Reagent Diluent to the working

concentration indicated on the C of A shipped with product. Detection antibodies may be stored at 2 – 8°C for up to 6 months. For long term storage, it is recommended to aliquot into working volumes and store at -70°C in a manual defrost the freezer. **Avoid repeated freeze and thaw cycles.**

RECOMBINANTS STANDARD

Recombinant Human BMP-2 Standard: Centrifuge vial prior to opening. Reconstitute each vial with 0.5 mL of Reagent Diluent. Refer to the lot-specific datasheet for amount supplied. The rProtein may be stored at 2 – 8°C for one (1) month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer. **Avoid repeated freeze and thaw cycles.**

STREPTAVIDIN

Streptavidin-HRP: Each vial contains approximately 2.0 mL of streptavidin horseradish-peroxidase (HRP). Dilute to the working concentration specified on the vial label using Reagent Diluent. Upon receipt, SteptAvidin-HRP conjugate should be stored at 2 – 8°C, **DO NOT FREEZE.**

SUBSTRATE

TMB Liquid Substrate "Ready to Use" (TMB Substrate should be at ambient temperature prior to use): 60.0 mL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" is provided. The high quality of the substrate can be preserved by storing at temperatures between 2 – 8ºC. When properly stored, TMB Microwell Substrate is stable for a minimum of 48 months from the manufactured date.

PLATE PREPARATION

1. Dilute the capture antibody to the working concentration in PBS without carrier protein and immediately add 100 µL to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300-400 µL of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300 µL block buffer to each well and incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

NOTE: Complete removal of liquid at each step is essential for good performance and sensitivity of assay.

ASSAY PROCEDURE

Standard/Sample: Add 100 µL of the working dilution with reagent dilution standard or sample to each well (duplicate recommended). Cover plate with an adhesive plate cover and incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Add 100 µL of the diluted in Reagent Diluent detection antibody per well. Cover with a new adhesive plate cover and incubate at room temperature for 2 hours.

StreptAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Add 100 µL of the working dilution (the dilution factor may require optimization) to each well. Cover and incubate at room temperature for 20-30 minutes. Exposure to direct light should be avoided.

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 µL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development. Exposure to direct light should be avoided.

Stop Solution: Add 50-100 µL of Stop Solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm.

PRODUCT USE LIMITATION

These products are intended for research use only.