APA014Hu02 10μg
Active Bone Morphogenetic Protein 4 (BMP4)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ser22~Arg408
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 9.0
Predicted Molecular Mass: 48.0kDa
Accurate Molecular Mass: 48kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8°C for one month.
Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

SHASLIPET GKKKVAEIQG HAGGRRSGQS
HELLRDFEAT LLQMFGLRRR PQPSKSAVIP DYMRDLYRLQ SGEEEEEQIH
STGLEYPERP ASRANTVRSF HHEEHLENIP GTSENSAFRF LFNLSSIPEN
EVISSAELRL FREQVDQGPD WERGFHRINI YEVMKPPAEV VPGHLITRLL
DTRLVHHNVT RWETFDVSPA VLRWTREKQP NYGLAIEVTH LHQTRTHQGQ
HVRISRSLPQ GSGNWAQLRP LLVTFGHDGR GHALTRRRRA KRSPKHHSQR
ARKKNKNCRR HSLYVDFSDV GWNDWIVAPP GYQAFYCHGD CPFPLADHLN
STNHAIVQTL VNSVNSSIPK ACCVPTELSA ISMLYLDEYD KVVLKNYQEM
VVEGCGCR

[ACTIVITY]

BMP4 (Bone morphogenetic protein 4) is a member of the bone morphogenetic protein family, which is involved in bone and cartilage development, specifically tooth and limb development and fracture repair. It has been proven that HJV (Hemojuvelin) acts as a coreceptor of BMPs, including BMP4; therefore, a binding ELISA assay was constructed to detect the association of HJV with BMP4. Briefly, BMP4 were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to HJV-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-BMP4 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution , wells were incubated 15-25 minutes at 37 ℃. Finally, add 50 μL stop solution to the wells and read at 450 nm immediately. The binding activity of BMP4 with HJV was shown in Figure 1 and this effect was in a dose dependent manner.

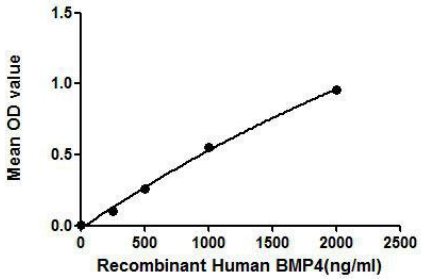

Figure 1. The binding activity of BMP4 with HJV.

To test the effect of BMP4 on cell apoptosis, HepG2 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 cells/well and allowed to attach overnight, then the medium was replaced with various concentrations of recombinant human BMP4 diluted with 5% serum standard DMEM. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37 ℃. Apoptosis of HepG2 cells after incubation with BMP4 for 72h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant human BMP4 for 72h. The result was shown in Figure 2. It was obvious that BMP4 significantly decreased cell viability of HepG2 cells. The ED50 of recombinant human BMP4 is 5.1 ug/ml.

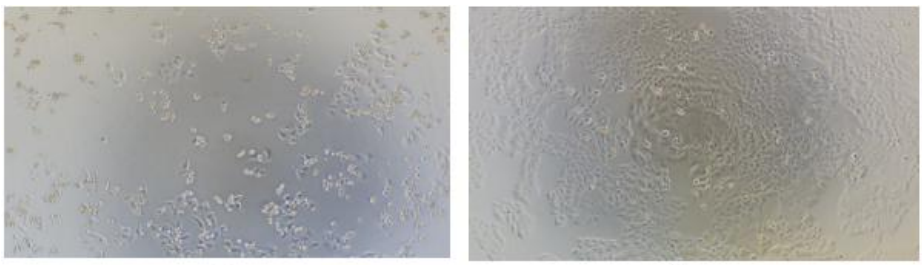

Figure 2. Inhibition of HepG2 cells proliferation after stimulated with recombinant human BMP4

(A) HepG2 cells cultured in DMEM, stimulated with 5 μg/mL BMP4 for 72h;
(B) Unstimulated HepG2 cells cultured in DMEM for 72h.

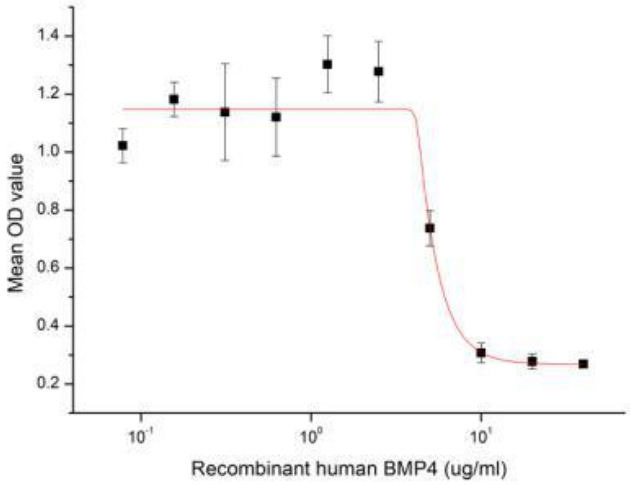

Figure 3. Inhibition of HepG2 cells proliferation after stimulated with recombinant human BMP4

[IDENTIFICATION]

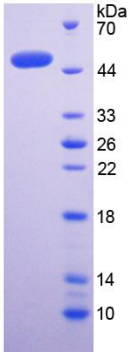

Figure 4. SDS-PAGE

Sample: Active recombinant BMP4, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.