APA014Ga01 10µg Active Bone Morphogenetic Protein 4 (BMP4) Organism Species: *Chicken (Gallus) 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: Ala244~Lys388 Tags: N-terminal His-tag Purity: >80% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose . Original Concentration: 2000µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.3 Predicted Molecular Mass: 17.8kDa Accurate Molecular Mass: 18kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) 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

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was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C 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.

[<u>SEQUENCE</u>]

AQTHQGK HVRISRSLPQ GHGGDWAQLR PLLVTFGHDG RGHALTRRAR RSPKHHGSRK NKKNCRRHAL YVDFSDVGWN DWIVAPPGYQ AFYCHGDCPF PLADHLNSTN HAIVQTLVNS VNSSIPKACC VPTELSAISM LYLDEYDK

[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. 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 chicken 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 $\,^\circ C$. 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 chicken 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 chicken BMP4 is 4.86 ug/ml.

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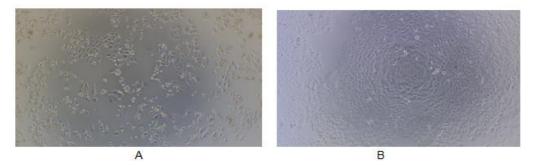


Figure 1. Inhibition of HepG2 cells proliferation after stimulated with recombinant chicken BMP4

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

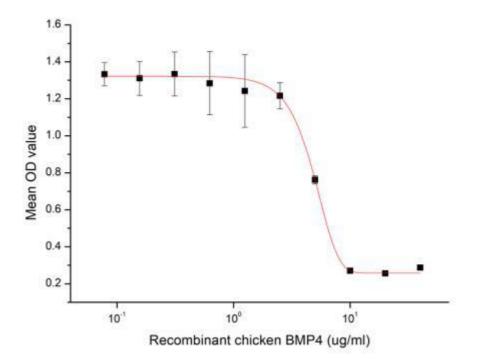


Figure 2. Inhibition of HepG2 cells proliferation after stimulated with recombinant chicken BMP4

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[IDENTIFICATION]

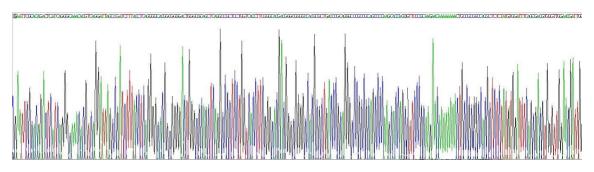


Figure 3. Gene Sequencing (extract)

kDa 70
44
33
26
22
18
14
10

Figure 4. SDS-PAGE

Sample: Active recombinant BMP4, Gallus

[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.