

**APA013Hu01 200µg**

**Active Active Bone Morphogenetic Protein 2 (BMP2)**

**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:** Gln283~Arg396

**Tags:** Two N-terminal Tags, His-tag and GST-tag

**Purity:** >98%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**Buffer Formulation:** 20mM Tris, 500mM NaCl, pH8.0, containing 1mM EDTA, 0.01% sarcosyl, 5%Trehalose and Proclin300.

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 7.3

**Predicted Molecular Mass:** 42.9kDa

**Accurate Molecular Mass:** 43kDa 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.

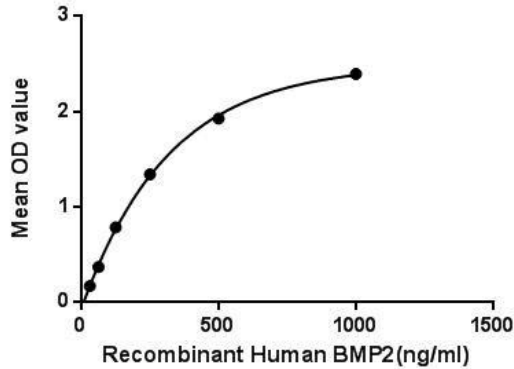
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration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

## **[ SEQUENCE ]**

QAKHKQRK RLKSSCKRHP  
LYVDFSDVGW NDWIVAPPGY HAFYCHGECF FPLADHLNST NHAIVQTLVN  
SVNSKIPKAC CVPTELSAIS MLYLDENEKV VLKNYQDMVV EGCGR

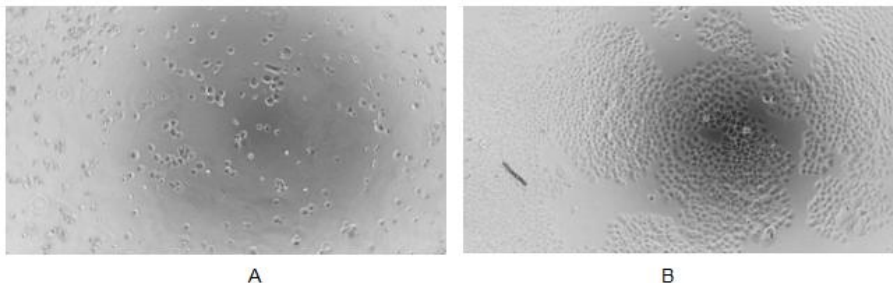
## **[ ACTIVITY ]**

Bone morphogenetic protein 2 (BMP2) belongs to the TGF- $\beta$  superfamily of proteins. It plays an important role in the development of bone and cartilage. It is involved in the hedgehog pathway, TGF beta signaling pathway, and in cytokine-cytokine receptor interaction. BMP2 is also involved in cardiac cell differentiation and epithelial to mesenchymal transition. Like many other proteins from the BMP family, BMP2 has been demonstrated to potently induce osteoblast differentiation in a variety of cell types. Besides, Noggin (NOG) has been identified as an interactor of BMP2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human BMP2 and recombinant human NOG. Briefly, BMP2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to NOG-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-BMP2 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°C. Finally, add 50 $\mu$ L stop solution to the wells and read at 450nm immediately. The binding activity of BMP2 and NOG was shown in Figure 1, and this effect was in a dose dependent manner.



**Figure 1. The binding activity of BMP2 with NOG.**

To test the effect of BMP2 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 BMP2 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  $\mu$ l 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 °C. Apoptosis of HepG2 cells after incubation with BMP2 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 BMP2 for 72h. The result was shown in Figure 2. It was obvious that BMP2 significantly decreased cell viability of HepG2 cells. The ED50 of recombinant human BMP2 is 3.25  $\mu$ g/ml.

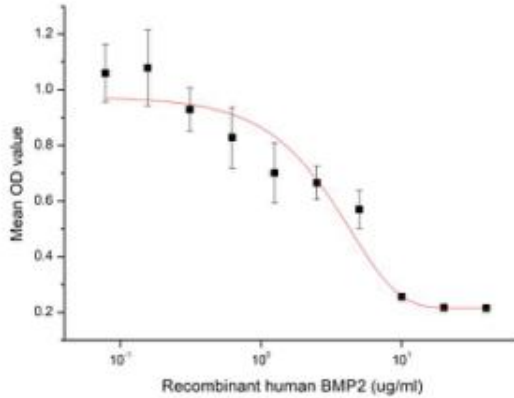


A

B

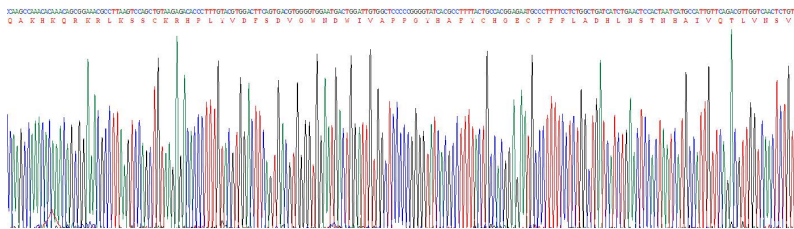
**Figure 2. Inhibition of HepG2 cells proliferation after stimulated with recombinant human BMP2**

- (A) HepG2 cells cultured in DMEM, stimulated with 5 µg/mL BMP2 for 72h;
- (B) Unstimulated HepG2 cells cultured in DMEM for 72h.

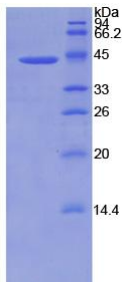


**Figure 3. Inhibition of HepG2 cells proliferation after stimulated with recombinant human BMP2**

**[ IDENTIFICATION ]**



**Figure 4. Gene Sequencing (Extract)**



**Figure 5. SDS-PAGE**

**Sample: Active recombinant BMP2, 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.