Coud-Clone Corp.

APJ226Hu03 100µg Active Biglycan (BGN) 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: Val49~Tyr366 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.5 Predicted Molecular Mass: 39.7kDa Accurate Molecular Mass: 40kDa 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.

Cloud-Clone Corp.

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.

[<u>SEQUENCE</u>]

VLDPDSVTPTYSAMCPFGCHCHLRVVQCSDLGLKSVPKEISPDTTLLDLQNNDISELRKDDFKGLQHLYAL VLVNNKISKIHEKAFSPLRKLQKLYISKNHLVEIPPNLPSSLVELRIHDNRIRKVPKGVFSGLRNMNCIEM GGNPLENSGFEPGAFDGLKLNYLRISEAKLTGIPKDLPETLNELHLDHNKIQAIELEDLLRYSKLYRLGLG HNQIRMIENGSLSFLPTLRELHLDNNKLARVPSGLPDLKLLQVVYLHSNNITKVGVNDFCPMGFGVKRAYY NGISLFNNPVPYWEVQPATFRCVTDRLAIQFGNY

[ACTIVITY]

Biglycan, also known as BGN, is a a small leucine-rich repeat proteoglycan (SLRP). It can be detected in a variety of extracellular matrix tissues, including bone, cartilage and tendon. Biglycan consists of a protein core containing leucine-rich repeat regions and two glycosaminoglycan (GAG) chains consisting of either chondroitin sulfate (CS) or dermatan sulfate (DS). Non-glycanated forms of biglycan (no GAG chains) increase with age in human articular cartilage. Biglycan interacts with collagen, both via the core protein and GAG chains. Biglycan plays a role in the mineralisation of bone. Biglycan core protein binds to the growth factors BMP-4 and influences its bioactivity. To test the effect of BGN on cell apoptosis, 3T3-L1 cells were seeded into triplicate wells of 96-well plates and allowed to attach overnight, then the medium was replaced with various concentrations of recombinant human BGN. 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 1h at 37 $^\circ\!\!\mathbb{C}$. Proliferation of 3T3-L1 cells after incubation with BGN 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

Cloud-Clone Corp.

human BGN for 72h. The result was shown in Figure 2. It was obvious that BGN significantly inhibit cell viability of 3T3-L1 cells. The ED50 is 0.89 μ g/mL.



Figure 1. Inhibition of 3T3-L1 cells proliferation after stimulated with BGN

- (A) 3T3-L1 cells cultured in DMEM, stimulated with 1.25 µg/ml BGN for 72h;
- (B) Unstimulated 3T3-L1 cells cultured in DMEM for 72h.



Figure 2. Inhibition of 3T3-L1 cells proliferation after stimulated with BGN.

Cloud-Clone Corp.

[IDENTIFICATION]

Figure 3. SDS-PAGE

Sample: Active recombinant BGN, Human

[<u>IMPORTANT NOTE</u>]

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.