## Cloud-Clone Corp.

APA222Hu01 50µg Active Interferon Beta (IFNb) 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: Met22~Asn187 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: 100µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.9 Predicted Molecular Mass: 23.7kDa Accurate Molecular Mass: 25kDa as determined by SDS-PAGE reducing conditions. [USAGE] Reconstitute in ddH<sub>2</sub>O to a concentration of 0.1-0.5 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.

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**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>]

MSYNLLGFL QRSSNFQCQK LLWQLNGRLE YCLKDRMNFD IPEEIKQLQQ FQKEDAALTI YEMLQNIFAI FRQDSSSTGW NETIVENLLA NVYHQINHLK TVLEEKLEKE DFTRGKLMSS LHLKRYYGRI LHYLKAKEYS HCAWTIVRVE ILRNFYFINR LTGYLRN

#### [ACTIVITY]

Interferon Beta (IFNb) is belongs to type I interferons (IFNs) family which a large subgroup of interferon proteins that help regulate the activity of the immune system. The IFNb proteins are produced in large quantities by fibroblasts. They have antiviral activity that is involved mainly in innate immune response. Two types of IFNb have been described, IFNb1 (IFNB1) and IFNb3 (IFNB3). IFNb1 is used as a treatment for multiple sclerosis as it reduces the relapse rate. To test the effect of IFNb on cell apoptosis, A549 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 5% serum standard DMEM prior to the addition of various concentrations of recombinant human IFNb. After incubated for 48h, 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 A549 cells after incubation with IFNb for 48h observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8(Cell Counting Kit-8) assay after incubation with recombinant IFNb for 48h. The result was shown in Figure2.

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It was obvious that IFNb significantly decreased cell viability of A549 cells. The

ED50 of recombinant human IFNb is 6.4µg/mL.



#### Figure 1. Cell apoptosis of A549 cells after stimulated with IFNb.

(A)A549 cells cultured in DMEM, stimulated with 5ug/ml IFNb for 48h;

(B)Unstimulated A549 cells cultured in DMEM for 48h.

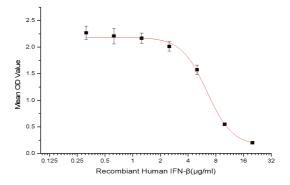
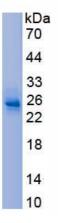


Figure 2. Cell apoptosis of A549 cells after stimulated with IFNb.

## [IDENTIFICATION]



#### Figure 3. SDS-PAGE

Sample: Active recombinant IFNb, 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.