APA222Mu01 10µg Active Interferon Beta (IFNb) Organism Species: *Mus musculus (Mouse) 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: Ile22~Asn182

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

Purity: >80%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 50µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 9.7

Predicted Molecular Mass: 49.7kDa

Accurate Molecular Mass: 40&25kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
- 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
- 5. Polymerization of the target protein: Dimerization, multimerization etc.

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Reconstitute in ddH_2O 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.

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]

INYKQLQLQ ERTNIRKCQE LLEQLNGKIN LTYRADFKIP MEMTEKMQKS YTAFAIQEML QNVFLVFRNN FSSTGWNETI VVRLLDELHQ QTVFLKTVLE EKQEERLTWE MSSTALHLKS YYWRVQRYLK LMKYNSYAWM VVRAEIFRNF LIIRRLTRNF QN

[<u>ACTIVITY</u>]

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 and allowed to attach, replaced with various concentrations of recombinant mouse IFNb. After incubated for 48 hours, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8).

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Briefly, 10 μ I 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 A549 cells after incubation with rmIFNb for 48 hours 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 mouse IFNb for 48 hours. The result was shown in Figure2. It was obvious that rmIFNb significantly decreased cell viability of A549 cells.

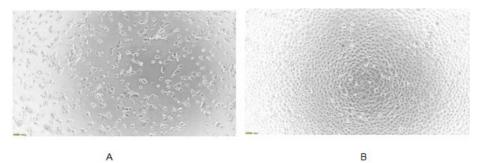
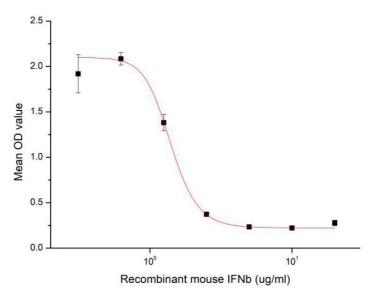
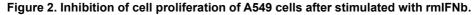


Figure 1. Inhibition of cell proliferation of A549 cells after stimulated with rmIFNb.

- (A) A549 cells cultured in DMEM, stimulated with 1.25 ug/ml rmIFNb for 48 hours;
- (B) Unstimulated A549 cells cultured in DMEM for 48 hours.





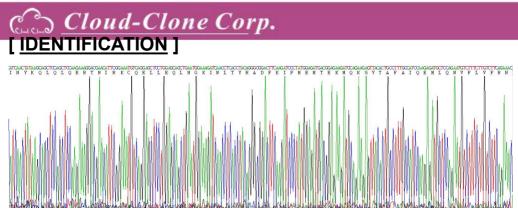


Figure 3. Gene Sequencing (extract)

kDa 70
44
33
26
22
18
14
10

Figure 4. SDS-PAGE

Sample: Active recombinant IFNb, Mouse

[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.