

APA222Ca61 100µg
Active Interferon Beta (IFNβ)
Organism Species: *Canis familiaris*; Canine (Dog)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ala21~Asn186

Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.2

Predicted Molecular Mass: 21.7kDa

Accurate Molecular Mass: 35kDa 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.

[USAGE]

Reconstitute in 10mM PBS (pH7.6) 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]

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AMSNDLLRSQ LSSSSLECQE LLLQLNGTTE  
YCLKDRINFE IP EEIEKSRQ FQKEDIILIT HEMFQKIFDI FRRNISRTGW  
NETTVENLLV KLHWQKEHLE IILEDVKEKE NFTWDNRTL L HLKYYLRIV  
QYLKAKEYSI CAWTIVQAEI CRNFFFLNIL TDYLQN
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[ACTIVITY]

Interferon Beta (IFN β) 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 IFN β proteins are produced in large quantities by fibroblasts. They have antiviral activity that is involved mainly in innate immune response. Two types of IFN β have been described, IFN β 1 (IFNB1) and IFN β 3 (IFNB3). IFN β 1 is used as a treatment for multiple sclerosis as it reduces the relapse rate. To test the effect of IFN β on cell apoptosis, A549 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 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 dog IFN β . After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ 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 A549 cells after incubation with IFN β 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 IFN β for 48h. The result was shown in Figure2. It was obvious that IFN β significantly decreased cell viability of A549 cells. The EC₅₀ of recombinant dog IFN β is 1.65 μ g/ml.

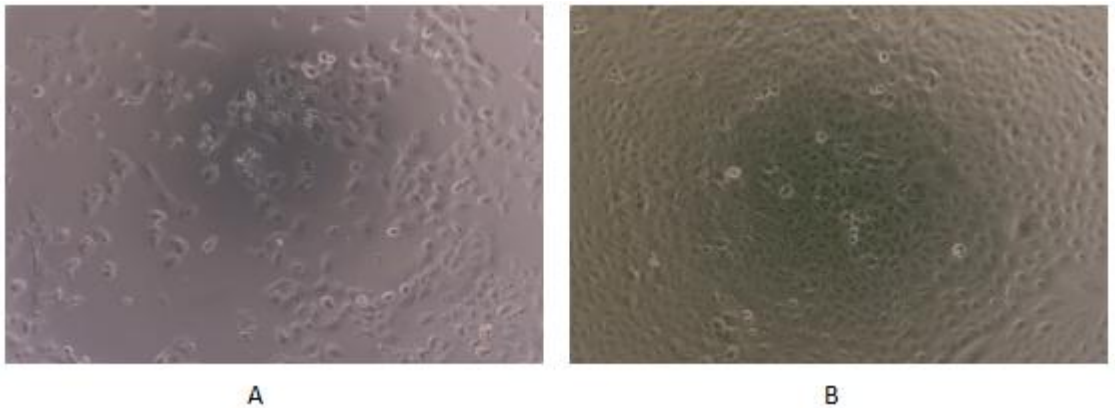


Figure 1. Cell apoptosis of A549 cells after stimulated with IFN β .

- (A) A549 cells cultured in DMEM, stimulated with 1.25 μ g/ml IFN β for 48h;
- (B) Unstimulated A549 cells cultured in DMEM for 48h.

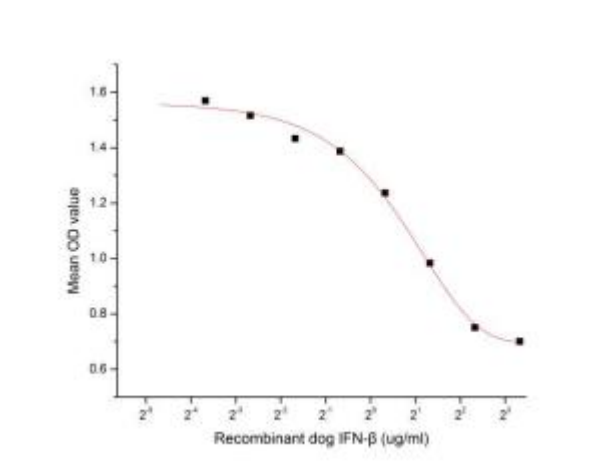


Figure 2. Cell apoptosis of A549 cells after stimulated with IFN β .

[IDENTIFICATION]

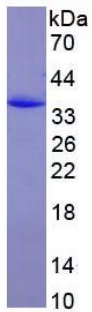


Figure 3. SDS-PAGE

Sample: Active recombinant IFN β , Dog

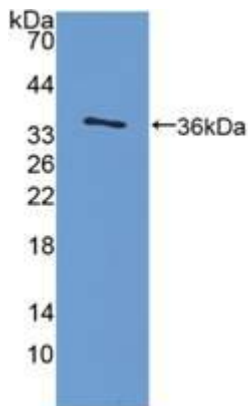


Figure 4. Western Blot

Sample: Recombinant IFN β , Dog;

Antibody: Rabbit Anti-dog IFN β Ab (PAA222Ca06)

[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.