

**APA222Ca01 100µg**  
**Active Interferon Beta (IFNβ)**  
**Organism Species: *Canis familiaris*; Canine (Dog)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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12th Edition (Revised in Aug, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ala21~Asn186

**Tags:** N-terminal His and GST Tag

**Purity:** >97%

**Traits:** Freeze-dried powder

**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:** 6.2

**Predicted Molecular Mass:** 50.1kDa

**Accurate Molecular Mass:** 47kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

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.

**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.

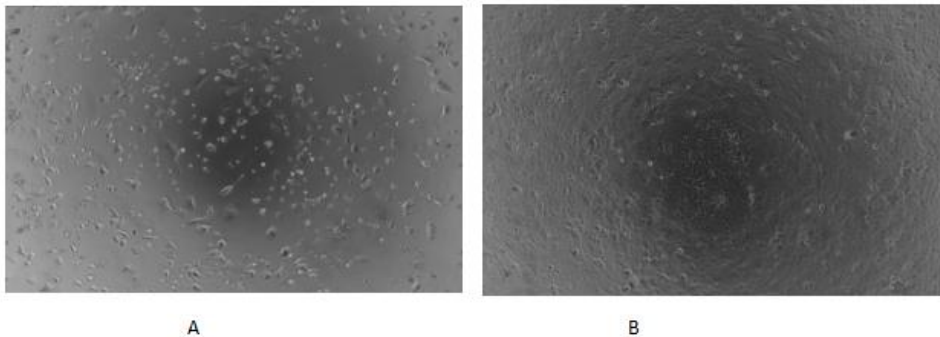
## **[ SEQUENCE ]**

```
          AMSNDLLRSQ LSSSSLECQE LLLQLNGTTE
YCLKDRINFE IP EEIEKSRQ FQKEDIILIT HEMFQKIFDI FRNISRGTGW
NETTVENLLV KLHWQKEHLE IILEDVKEKE NFTWDNRLL HLKYYLRIV
QYLKAKEYSI CAWTIVQAEI CRNFFFLNIL TDYLQN
```

## **[ ACTIVITY ]**

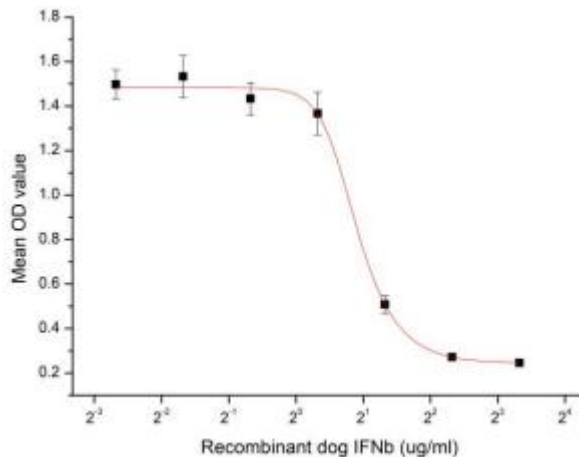
Interferon Beta (IFN $\beta$ ) is belongs to type I interferons (IFNs) family which a large subgroup of interferon proteins that help regulate the activity of the immune system. The IFN $\beta$  proteins are produced in large quantities by fibroblasts. They have antiviral activity that is involved mainly in innate immune response. Two types of IFN $\beta$  have been described, IFN $\beta$ 1 (IFNB1) and IFN $\beta$ 3 (IFNB3). IFN $\beta$ 1 is used as a treatment for multiple sclerosis as it reduces the relapse rate. To test the effect of IFN $\beta$  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 overnight, then the medium was replaced with various concentrations of recombinant dog IFN $\beta$  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 A549 cells after

incubation with IFN $\beta$  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 dog IFN $\beta$  for 72h. The result was shown in Figure 2. It was obvious that IFN $\beta$  significantly decreased cell viability of A549 cells. The EC<sub>50</sub> of recombinant dog IFN $\beta$  is 1.86  $\mu$ g/ml.



**Figure 1. Cell apoptosis of A549 cells after stimulated with recombinant dog IFN $\beta$ .**

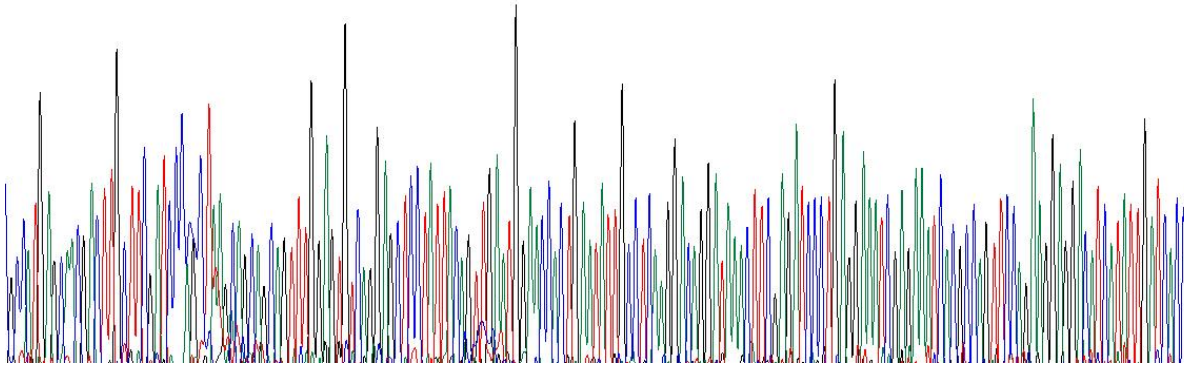
- (A) A549 cells cultured in DMEM, stimulated with 2.5  $\mu$ g/ml IFN $\beta$  for 72h;
- (B) Unstimulated A549 cells cultured in DMEM for 72h.



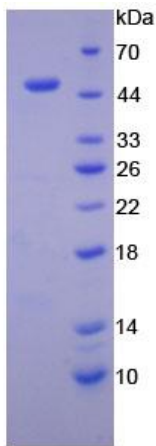
**Figure 2. Cell apoptosis of A549 cells after stimulated with recombinant dog IFN $\beta$ .**

## [ IDENTIFICATION ]

TGCGATGAGGAAAGACTTGTCTTTGATCCGAGCTAGGCGGCGAGTTTGGAGTGTGAGGAGCTCCATTATGAGTTGAAATGGACACCTGAAATTTGCCAAGGAGGAGATTAAC TTCGAGATCCCTGAGGAAATCGAGAAATCACCCGTTCCGAGGAGGACATCATATTGATCAC  
A M S N D L L R S Q L S S S S L E C Q E L L L Q L H G T T E Y C L K D R I N F E I P E E I E K S R Q F Q K E D I I L I T



**Figure 3. Gene Sequencing (extract)**



15% SDS-PAGE

**Figure 4. SDS-PAGE**

**Sample: Active recombinant IFN $\beta$ , Dog**

## [ 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.