#### APA222Ca02 100µg Active Interferon Beta (IFNb) Organism Species: *Canis familiaris; Canine (Dog)* Instruction manual

#### FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

#### [PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Ala21~Asn186 Tags: N-terminal His-tag Purity: >90% 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: 23.8kDa Accurate Molecular Mass: 24kDa 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.

**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.

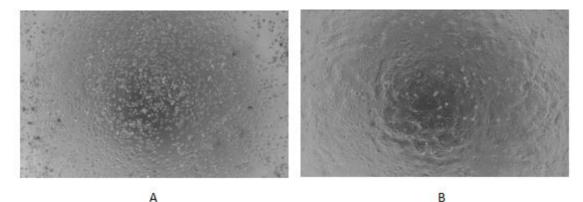
#### [SEQUENCE]

AMSNDLLRSQ LSSSSLECQE LLLQLNGTTE YCLKDRINFE IPEEIEKSRQ FQKEDIILIT HEMFQKIFDI FRRNISRTGW NETTVENLLV KLHWQKEHLE IILEDVKEKE NFTWDNRTLL HLKKYYLRIV QYLKAKEYSI CAWTIVQAEI CRNFFFLNIL TDYLQN

### [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 4,000 cells/well and allowed to attach overnight, then the medium was replaced with various concentrations of recombinant dog IFNb 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  $^{\circ}$ C. Apoptosis of A549 cells after

incubation with IFNb 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 IFNb for 72h. The result was shown in Figure 2. It was obvious that IFNb significantly decreased cell viability of A549 cells. The EC50 of recombinant dog IFNb is about  $3 \mu g/ml$ .





- (A) A549 cells cultured in DMEM, stimulated with 5 ug/ml IFNb for 72h;
  - (B) Unstimulated A549 cells cultured in DMEM for 72h.

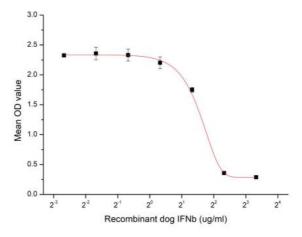


Figure 2. Cell apoptosis of A549 cells after stimulated with recombinant dog IFNb.

## [ IDENTIFICATION ]

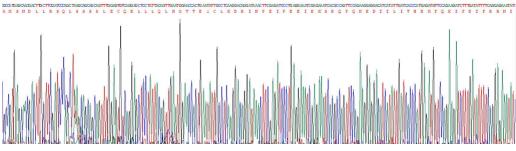


Figure 3. Gene Sequencing (extract)

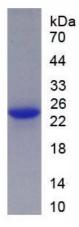


Figure 4. SDS-PAGE

Sample: Active recombinant IFNb, 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.