APA033Ga01 100μg Active Interferon Alpha (IFNa) Organism Species: *Chicken (Gallus) 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: Leu47~Thr191 Tags: N-terminal His and GST Tag Purity: >95% 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: 6.8 Predicted Molecular Mass: 47.0kDa Accurate Molecular Mass: 47kDa 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.

[<u>SEQUENCE</u>]

LQLL RDMAPTLPQL CPQHNASCSF NDTILDTSNT RQADKTTHDI LQHLFKILSS PSTPAHWNDS QRQSLLNRIH RYTQHLEQCL DSSDTRSRTR WPRNLHLTIK KHFSCLHTFL QDNDYSACAW EHVRLQARAW FLHIHNLTGN T

[ACTIVITY]

Interferon-alpha (IFNa), also known as leukocyte interferon, represents a group of related but distinct proteins that share over 95% amino acid sequence homology. They are members of the type I interferon family which share a common cell surface receptor composed of two subunits. IFN- α has both anti-viral and immunomodulatory activities on target cells. To test the effect of IFNa 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 chicken IFNa 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 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 °C. Apoptosis of A549 cells after incubation with IFNa 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 chicken IFNa for 72h. The result was shown in Figure 2. It was obvious that IFNa significantly decreased cell viability of A549 cells. The ED50 of recombinant chicken IFNa is 5.13 ug/ml.



Figure 1. Inhibition of A549 cells proliferation after stimulated with recombinant pig IFNa (A) A549 cells cultured in DMEM, stimulated with 5 μ g/mL IFNa for 72h; (B) Unstimulated A549 cells cultured in DMEM for 72h.

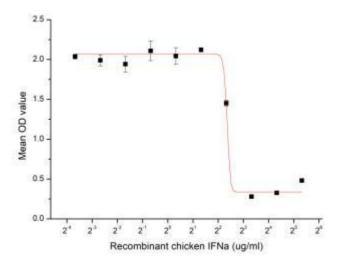


Figure 2. Inhibition of A549 cells proliferation after stimulated with recombinant chicken IFN-α.

[IDENTIFICATION]

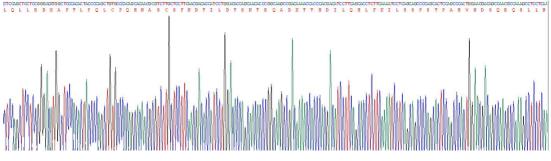


Figure 3. Gene Sequencing (extract)

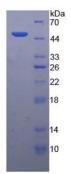


Figure 4. SDS-PAGE Sample: Active recombinant IFNa, Gallus

[<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.