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APA049Po61 10µg Active Interferon Gamma (IFNg) Organism Species: *Sus scrofa; Porcine (Pig) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell

Residues: Ser21~Lys166

Tags: N-terminal His-tag

Purity: >90%

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

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

Original Concentration: 100µg/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: 17.7kDa

Accurate Molecular Mass: 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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[<u>USAGE</u>]

Reconstitute in ddH₂O 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.

[<u>SEQUENCE</u>]

SYCQAPFFKE ITILKDYFNA STSDVPNGGP LFLEILKNWK EESDKKIIQS QIVSFYFKFF EIFKDNQAIQ RSMDVIKQDM FQRFLNGSSG KLNDFEKLIK IPVDNLQIQR KAISELIKVM NDLSPRSNLR KRKRSQTMFQ GQRASK

[ACTIVITY]

IFN-g is a dimerized soluble cytokine that is the only member of the type II class of interferons. The importance of IFNg in the immune system stems in part from its ability to inhibit viral replication directly and most importantly from its immunostimulatory and immunomodulatory effects. IFN-gamma dimers bind to IFN-gamma RI (alpha subunits) which then interact with IFN-gamma RII (beta subunits) to form the functional receptor complex of two alpha and two beta subunits. Inclusion of IFN-gamma RII increases the binding affinity for ligand and the efficiency of signal transduction. A functional binding ELISA assay was conducted to detect the interaction of recombinant pig IFN-g and recombinant human IFNgR1. Briefly, IFN-g was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to IFNgR1-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated

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for 1h with anti-IFN-g pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant pig IFN-g and recombinant human IFNgR1 was shown in Figure 1, the EC50 for this effect is 0.38 ug/mL.

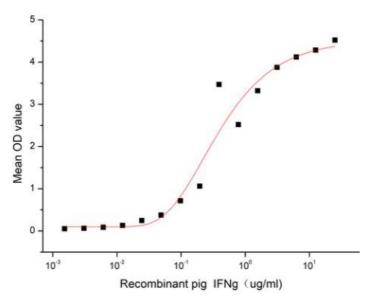
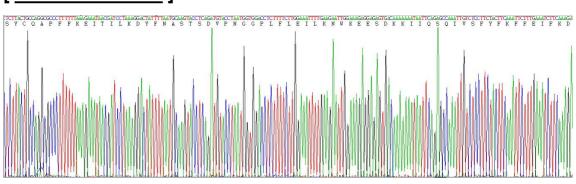


Figure 1. The binding activity of recombinant pig IFN-g and recombinant human IFNgR1



[IDENTIFICATION]

Figure 2. Gene Sequencing (extract)

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	70
1	44
	33
	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant IFNg, Pig

[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.