

APA049Ra01 100µg

Active Interferon Gamma (IFNg)

Organism Species: Rattus norvegicus (Rat)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Gln23~Cys156
Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA,

1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300. **Applications:** Cell culture; Activity Assays; In vivo assays.

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

Predicted isoelectric point: 9.3

Predicted Molecular Mass: 16.8kDa

Accurate Molecular Mass: 16kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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.



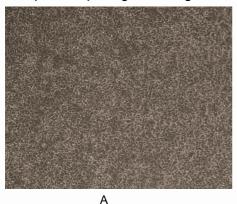
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

QGTLIESL ESLKNYFNSS SMDAMEGKSL LLDIWRNWQK DGNTKILESQ IISFYLRLFE VLKDNQAISN NISVIESHLI TNFFSNSKAK KDAFMSIAKF EVNNPQIQHK AVNELIRVIH QLSPESSLRK RKRSRC

[ACTIVITY]

Interferon gamma (IFNγ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. The importance of IFNγ in the immune system stems in part from its ability to inhibit viral replication directly, and most importantly from its immunostimulatory and immunomodulatory effects. It has been reported that IFN-γ promotes production of inducible Nitric Oxide Synthase (iNOS) in macrophages as an important activator. After stimulated with IFN-γ, morphological changes will occur in murine macrophage cell line (Raw 264.7 cells), and inducible nitric-oxide synthase (iNOS) in the cells will increase. Raw 264.7 cells were incubated in DMEM with IFN-γ (10ng/mL) for 24h, then cells were observed by inverted microscope and iNOS in cell lysates was detected by ELISA. Effect of IFN-γ on morphological change of Raw 246.7 cells was shown in Figure 1.



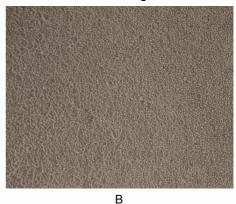


Figure 1. Morphological change of Raw 264.7cells after stimulation of IFNg.



- (A) Raw 264.7 cells cultured in DMEM, stimulated with IFNg;
- (B) Unstimulated Raw 264.7 cells cultured in DMEM (negative control).

Effect of IFN-γ on the expression of iNOS was shown in Table 1.

Table 1. ELISA detection of iNOS expression from RAW 246.7 cells stimulated by IFNg.

Sample	O.D. value	Corrected	Concentration of iNOS
(cell lysates of Raw 264.7 cells)	2.24	2.22	(ng/mL)
stimulated with IFN-γ (10ng/mL)	3.31	3.22	40.97
unstimulated	0.37	0.28	3.66

[IDENTIFICATION]

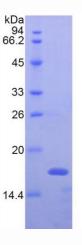


Figure 2. SDS-PAGE

Sample: Active recombinant IFNg, Rat

Coud-Clone Corp.

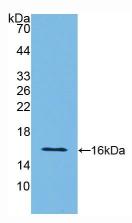


Figure 3. Western Blot

Sample: Recombinant IFNg, Rat;

Antibody: Rabbit Anti-Rat IFNg Ab (PAA049Ra01)