Cloud-Clone Corp.

APA049Ra01 200µg 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

1st Edition (Apr, 2016)

#### [PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Gln23~Cys156 Tags: N-terminal His-tag Purity: >95% Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 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

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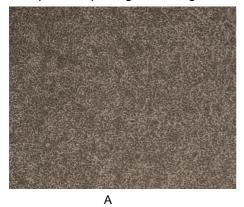
protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

# [SEQUENCE]

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

### [ACTIVITY]

Interferon gamma (IFN $\gamma$ ) is a dimerized soluble cytokine that is the only member of the type II class of interferons. The importance of IFN $\gamma$  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- $\gamma$  promotes production of inducible Nitric Oxide Synthase (iNOS) in macrophages as an important activator. After stimulated with IFN- $\gamma$ , 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- $\gamma$  (10ng/mL) for 24h, then cells were observed by inverted microscope and iNOS in cell lysates was detected by ELISA. Effect of IFN- $\gamma$  on morphological change of Raw 246.7 cells was shown in Figure 1.



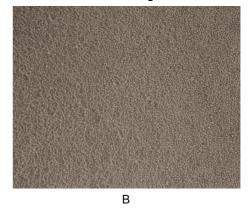


Figure 1. Morphological change of Raw 264.7cells after stimulation of IFNg.

- (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- $\gamma$  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<br>(cell lysates of Raw 264.7 cells) | O.D. value | Corrected | Concentration of iNOS<br>(ng/mL) |
|---|------------|-----------|----------------------------------|
| stimulated with IFN-γ (10ng/mL)             | 3.31       | 3.22      | 40.97                            |
| unstimulated                                | 0.37       | 0.28      | 3.66                             |

### [IDENTIFICATION]

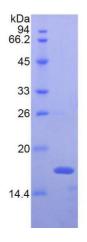


Figure 2. SDS-PAGE

Sample: Active recombinant IFNg, Rat

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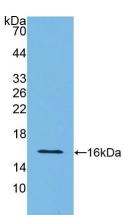


Figure 3. Western Blot Sample: Recombinant IFNg, Rat; Antibody: Rabbit Anti-Rat IFNg Ab (PAA049Ra01)

# [IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.