

## Human IFN-gamma (PeliKine™) ELISA Kit

Catalog No. M1933

3 x 96 tests

### INTRODUCTION

IFN gamma is produced during an immune response by CD8+, NK, gamma delta and TH1 T helper cells. It differs structurally and functionally from IFN alpha and IFN beta; binds to distinct receptors and has pronounced immuno-regulatory effects, including activation of macrophages to enhance phagocytosis and tumor killing capability, activation and growth enhancement of cytolytic T-cells and NK-cells, and induction of class II MHC antigen and Fc gamma receptor on macrophages and many other cell types.

IFN gamma also regulates humeral immune responses: it induces immunoglobulin secretion by activated B-cells stimulated with IL-2 and potentiates IL-4 induced proliferation of human B-cells. IFN gamma has documented antiviral and antiprotozoal activities, although IFN alpha and IFN beta seem to have more potent antiviral activities than IFN gamma. Several substances originally described for their biological activities have been identified as IFN gamma; macrophage activating factor (MAF), T-cell replacing factor (TRF), Type II interferon and immune interferon.

Bioassays for the quantification of IFN $\gamma$ , based on cytopathic reductive effects of IFN gamma on cultured cells have been used for several years. In this assay IFN gamma reduces the killing of a target cell line such as L929 (mouse), HEp2C or A549 (human) cells by for example, encephalomyocarditis virus. An alternative assay system involves measurement of induction of HLA-DR antigens on tumor cells, which can be detected in a cell ELISA. These assays, although sensitive, are time consuming and might be susceptible to interference by other substances.

The PeliKine (compact)™ human IFN gamma ELISA kit has been developed for faster, more reproducible and specific quantification of human IFN gamma in serum, plasma and other body fluids, as well as in cell-culture supernatant.

### KIT COMPONENT LIST

Quantity	Kit component		Volume	Cap :
1 vial	coating antibody	100-fold concentrated	375 $\mu$ l	red
1 vial	blocking reagent	50-fold concentrated	2 ml	transparent
2 vial	IFN $\gamma$ standard (lyophilised)	see label	500 $\mu$ l	black
1 vial	biotinylated antibody	100-fold concentrated	375 $\mu$ l	yellow
1 vial	streptavidin-poly-HRP conjugate	10,000-fold concentrated	20 $\mu$ l	brown
1 bottle	HPE-dilution buffer	5- fold concentrated	60 ml	
3 pcs	microtiter plate + lid	-	-	
10 pcs	plate seals	-	-	



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## **STORAGE AND STABILITY**

The Pelikine compact™ ELISA kit should be stored at -18°C to -32°C. The performance of the kit is guaranteed until the expiration date shown on the case label.

## **SPECIFICITY**

No cross reactivity was observed with the following recombinant human proteins: IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, Macrophage Colony Stimulating Factor (M-CSF), Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Leukemia Inhibitory Factor (LIF), RANTES, Stem Cell Factor/ Mast Cell Factor (SCF/MCF), Transforming Growth Factor  $\beta$ -1 (TGF $\beta$ -1), Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ) and Tumor Necrosis Factor  $\beta$  (TNF $\beta$ /Lymphotoxin).

## **STANDARD**

A natural human IFN $\gamma$  standard has been calibrated against the WHO reference preparation (IFN $\gamma$  88/606; National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, U.K. 1 WHO Unit = 53 pg IFN $\gamma$ ).

The kit contains one lyophilized vial natural human IFN $\gamma$

Reconstitute one lyophilized standard by adding 500  $\mu$ l of distilled water to the vial. Incubate for 10 minutes at room temperature and mix gently. After reconstitution the standard must be kept cold (2-8°C) and stored frozen after use (<-18°C, preferably <-70°C).

## **STANDARD CURVE**

Label 7 tubes, one tube for each dilution: 500, 200, 80, 32, 12.8, 5.1, and 2.0 pg/ml. Pipette 600  $\mu$ l of working-strength HPE-dilution buffer into the tube labeled 500 pg/ml and 300  $\mu$ l of working-strength HPE-dilution buffer into the other tubes. Transfer 75  $\mu$ l of the IFN $\gamma$  standard (4500 pg/ml) into the first tube labeled 500 pg/ml, mix well and transfer 200  $\mu$ l of this dilution into the second tube labeled 200 pg/ml. Repeat the serial dilutions six more times by adding 200  $\mu$ l of the previous tube of diluted standard to the 300  $\mu$ l of dilution buffer. Label one tube 0 pg/ml (working-strength HPE-dilution buffer)

It is recommended to prepare two separate series for each assay.

## **SAMPLES**

It is recommended to dilute the test samples at least 1:2 in working-strength HPE-dilution buffer. If high levels of IFN $\gamma$  (outside the standard curve) are expected in the test samples, additional dilutions of sample i.e. 1:10 and 1:50 should also be prepared.

## **SENSITIVITY**

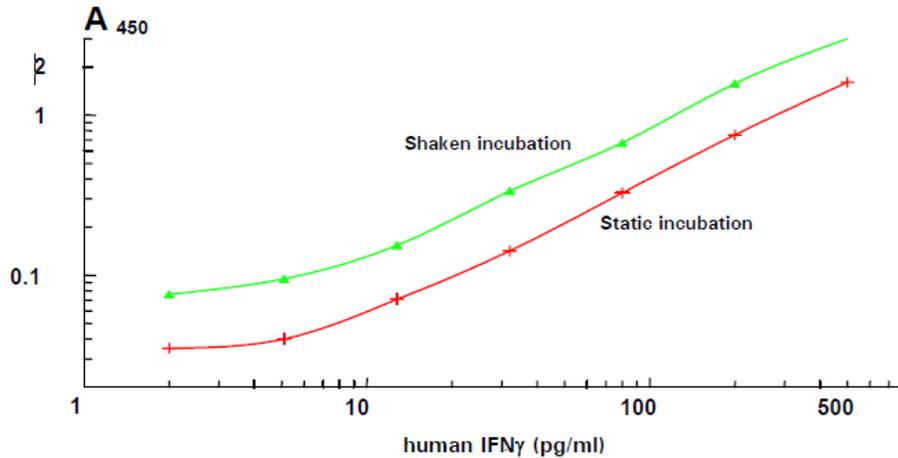
MEAN calculated zero signal + 3 SD : 1-2 pg/ml (shake - static incubation)  
2 x (MEAN calculated zero signal) : 4-6 pg/ml (shake - static incubation)



## EXPECTED VALUES

IFN $\gamma$  values in fresh serum and plasma samples of healthy individuals are below 10 pg/ml.

## TYPICAL STANDARD CURVE



	STATIC INCUBATION	SHAKEN INCUBATION
	Calculated mean absorbance at 450 nm	
substrate blank	0	0
0 pg/ml	0.025	0.073
2.0 pg/ml	0.035	0.074
5.1 pg/ml	0.040	0.095
12.8 pg/ml	0.071	0.154
32 pg/ml	0.142	0.336
80 pg/ml	0.327	0.671
200 pg/ml	0.753	1.572
500 pg/ml	1.604	> 3.000

**DO NOT USE THESE DATA TO CONSTRUCT A STANDARD CURVE FOR SAMPLE VALUE CALCULATIONS**

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**



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