Human IFN-gamma Sandwich ELISA Kit Datasheet

For the quantitative detection of human IFN-gamma concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00063
Product Name	Human IFN-gamma Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3458
SwissProt	P01579

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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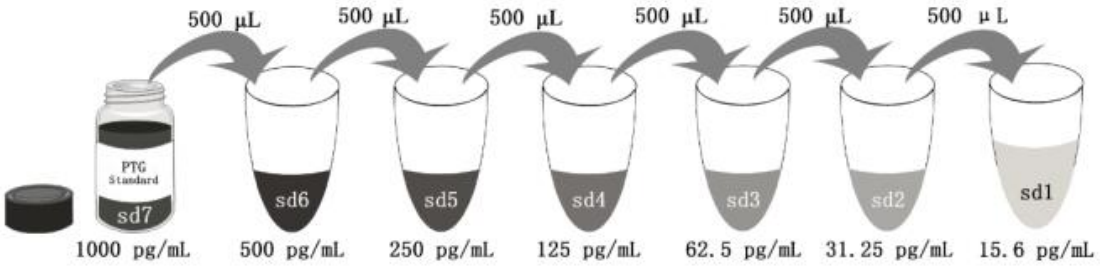
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00063 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IFN-gamma ELISA kit is to be used to detect and quantify protein levels of endogenous IFN-gamma. The assay recognizes human IFN-gamma. An antibody specific for IFN-gamma has been pre-coated onto the microwells. The IFN-gamma protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IFN-gamma is added to detect the captured IFN-gamma protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Interferon gamma (IFNG) is a soluble cytokine that is the only member of the type II class of interferons. It is secreted by Th1 cells, cytotoxic T cells and NK cells. The cytokine is associated with antiviral, immunoregulatory and anti-tumor properties and is a potent activator of macrophages. It plays crucial roles in pathogen clearance. Aberrant IFNG expression is associated with a number of autoinflammatory and autoimmune diseases. It has been identified in many studies as a biomarker for pleural tuberculosis (TB). Mutations in this gene are associated with aplastic anemia.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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Safety Notes

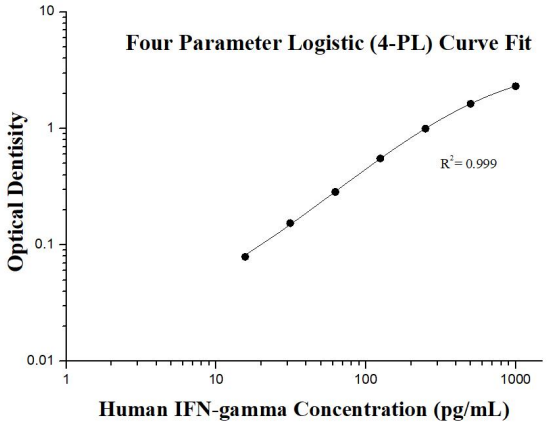
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.035 0.032	0.0335	-
15.63	0.112 0.113	0.1125	0.079
31.25	0.183 0.192	0.1875	0.154
62.5	0.322 0.315	0.3185	0.285
125	0.56 0.614	0.587	0.5535
250	1.01 1.056	1.033	0.9995
500	1.687 1.647	1.667	1.6335
1000	2.317 2.375	2.346	2.3125

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	688.0	38.0	5.5
2	20	202.4	14.8	7.3
3	20	51.0	3.5	6.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	927.0	79.0	8.5
2	24	259.7	20.7	8.0
3	24	66.0	4.2	6.4

Recovery

The recovery of IFN-gamma spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	92	86-101
	1:4	98	92-102
Cell culture supernatants	1:2	118	110-121
	1:4	104	84-113

Sample Values

Cell Culture supernatants - Human peripheral blood mononuclear cells (PBMC) (5 × 10^5 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. The cell culture supernatants were stimulated for different conditions and assayed for human IFN-gamma. (*** Day 1 : PBMC were stimulated used by 10 ug/mL PHA 1 day and 50 ng/mL LPS 2 hours; Day 3: PBMC were stimulated used by 10ug/mL PHA 2 days and 50ng/mL LPS 1 day; Day 5: PBMC were stimulated used by 10 ug/mL PHA 3 days and 50ng/mL LPS 2 days**)

Stimulated conditions	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
PHA 10ug/mL	129	305	371
PHA 10ug/mL+ LPS 50ng/mL *	253	372	263
Unstimulated	ND	ND	ND

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Sensitivity

The minimum detectable dose of human IFN-gamma is 1.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of IFN-gamma in various matrices and diluted with the appropriate **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	82	112
	Range (%)	80-86	102-125
1:4	Average% of Expected	94	115
	Range (%)	87-101	113-117
1:8	Average% of Expected	95	112
	Range (%)	89-104	106-121
1:16	Average% of Expected	99	115
	Range (%)	91-105	110-122

Calibration

This immunoassay is calibrated against a highly purifed *E. coli*-expressed recombinant human IFN-gamma produced at Proteintech Systems.

NIBSC/WHO International Standard for Interferon gamma (82/587), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard(82/587) parallels the Proteintech standard curve. To convert sample values obtained with the Human Interferon gamma ELISA kit to approximate NIBSC (82/587) units, use the equation below.

NIBSC (82/587) approximate value (IU/m L) = 6.31 x Proteintech Human Interferon gamma value (pg/mL)

References

1. Gray PW. et al. (1982). Nature. 298:859-63.
2. Schoenborn JR. et al. (2007). Adv Immunol. 96:41-101.
3. Denkinger CM. Et al. (2013). PLoS One.8:e85447.
4. Schroder, K. et al. (2004). J Leukoc Biol. 75:163-89.

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