Rat IFN-gamma Sandwich ELISA Kit Datasheet

For the quantitative detection of rat IFN-gamma concentrations in cell culture supernatants.

General Information

Catalogue Number	KE20007
Product Name	Rat IFN-gamma Sandwich ELISA Kit
Species cross-reactivity	Rat
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	25712
SwissProt	P01581

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 - 8000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100×) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial	1 vial	
Sample Diluent PT 1-eg - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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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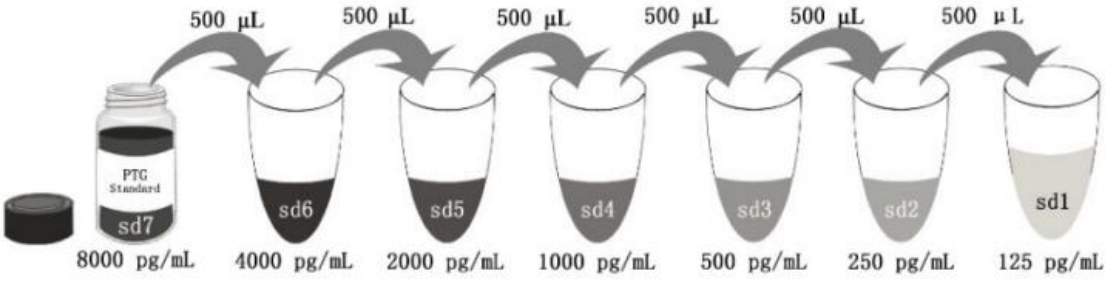
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-eg is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 1-eg in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.



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-eg	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE20007 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 rat 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

The IFNs were originally discovered as agents that interfere with viral replication. Initially, they were classified by the secreting cell type but are now classified into type I and type II according to receptor specificity and sequence homology. 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 samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested

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according to the individual samples.

Safety Notes

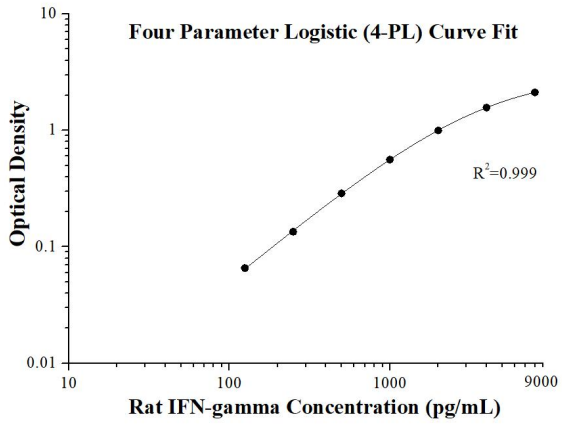
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.098 0.101	0.1	-
125	0.166 0.164	0.165	0.066
250	0.227 0.241	0.234	0.135
500	0.39 0.385	0.388	0.288
1000	0.667 0.652	0.660	0.560
2000	1.102 1.095	1.099	0.999
4000	1.697 1.643	1.67	1.571
8000	2.254 2.186	2.22	2.121

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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	1,885.8	44.9	2.4
2	20	895.5	25.6	2.9
3	20	202.0	11.0	5.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,944.0	81.8	4.2
2	24	934.9	47.8	5.1
3	24	206.3	18.2	8.8

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 (%)
Cell culture supernatants	1:30	82	78-89
	1:60	104	78-128

Sample Values

Rat splenocytes (1 x10^7cells/mL) were cultured for 5 days in DMEM plus 10% fetal bovine serum and stimulated with 5.0 µg/mL Concanavalin A. An aliquot of cell culture supernate was removed, assayed for rat IFN-γ and measured:

Condition	(ng/mL)
Unstimulated	-
Stimulated for 5d	28

Sensitivity

The minimum detectable dose of rat IFN-gamma is 3.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, three samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The samples Cell culture supernatants were initially diluted 1:2)

		Cell culture supernatants
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	99
	Range (%)	95-102
1:8	Average% of Expected	99
	Range (%)	94-106
1:16	Average% of Expected	92
	Range (%)	87-94

References

1. Isaacs, A. et al.(1957) The interferon Proc. R. Soc. Lond. B Biol. Sci. 147: 258-267.
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4. Wang Z. et al.(2014) Biosci Biotechnol Biochem.78: 588-92.
5. Chegou NN. et al.(2012)PLoS One.7(6):e38501
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