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Antibodies | ELISA kits | Proteins

AuthentiKine™

Human IFN alpha 2A Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IFN alpha 2A in cell culture supernatants.

General Information

Catalogue Number	KE00207
Product Name	AuthentiKine™ Human IFN alpha 2A Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3440
SwissProt	

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants.	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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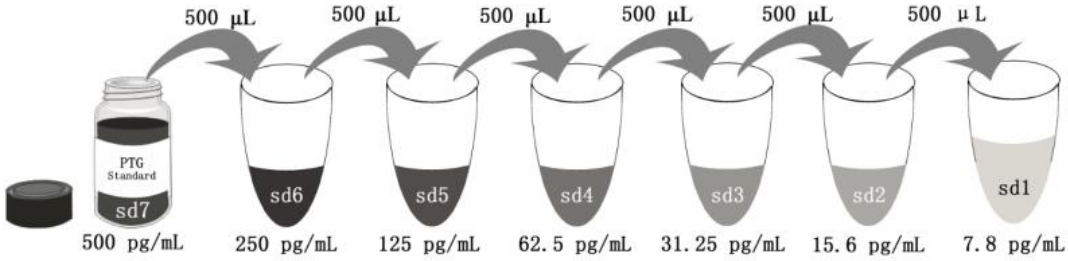
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



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

KE00207 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IFN alpha 2A ELISA kit is to be used to detect and quantify protein levels of endogenous IFN alpha 2A. The assay recognizes human IFN alpha 2A. An antibody specific for IFN alpha 2A has been pre-coated onto the microwells. The IFN alpha 2A protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IFN alpha 2A is added to detect the captured IFN alpha 2A protein. For signal development, 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

Human interferon alpha-2 (IFNA2), is a member of the Type I interferon cytokine family, known for its antiviral and anti-proliferative functions. Several IFN alpha2 alleles have been described, and the best known are alpha-2a and alpha-2b. IFN alpha2 binds a plasma membrane receptor made of IFNAR1 and IFNAR2 that is ubiquitously expressed, and thus is able to act on virtually all body cells. interferon alpha 2 is effective in reducing the symptoms and duration of the common cold and in treating many types of cancer, including some hematological malignancies and solid tumors. Interferon alpha-2 products, such as interferon alpha-2a (IFNA2) are used to treat multiple diseases such as hairy cell leukemia and hepatitis c.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for Cell culture

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supernatants.

Safety Notes

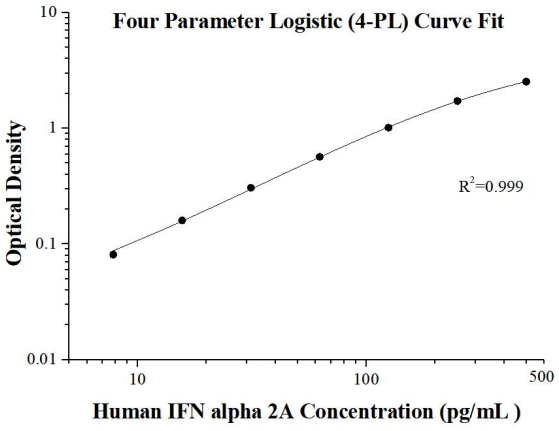
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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
3	Diluent Detection antibody, HRP-conjugated 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
4	Stop Solution	100 µL	0 min	Do not wash	-
5	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	0.094 0.096	0.095	0
7.8	0.177 0.175	0.176	0.081
15.6	0.256 0.254	0.255	0.160
31.2	0.401 0.401	0.401	0.306
62.5	0.659 0.664	0.662	0.567
125	1.108 1.111	1.110	1.015
250	1.800 1.838	1.819	1.724
500	2.637 2.625	2.631	2.536

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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	238.7	3.5	1.4
2	20	55.2	0.8	1.5
3	20	13.1	0.6	4.3

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	231.1	4.4	1.9
2	24	53.8	1.0	1.9
3	24	13.7	0.7	5.2

Recovery

The recovery of IFN alpha 2A 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	1:2	95	85-102
	1:4	96	90-104

Sample Values

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were unstimulated or stimulated with 50 μg/mL poly I:C in the presence of 10ug/ml Lipofectamine 2000 for 24h . Aliquots of the cell culture supernates were removed and assayed for levels of human IFN alpha 2A.

Condition	Day1 (pg/mL)
Unstimulated	-
Stimulated	158.5

Sensitivity

The minimum detectable dose of human IFN alpha2A is 0.10 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	103
	Range (%)	102-104
1:8	Average% of Expected	106
	Range (%)	103-108
1:16	Average% of Expected	118
	Range (%)	110-128

References

1. Franciane Paul. et al. (2015). Gene.567(2):132-7.
2. A Kaser. et al.(2001) Cell Mol Biol.47(4):609-17.
3. J Hiscott. et al.(1984) Nucleic Acids Res.12(9): 3727-46.
4. John Kirkwood. et al. (2002) Semin Oncol. 29(3 Suppl 7):18-26.

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