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AuthentiKine™

Human IFN alpha 2A Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IFN alpha 2A in serum, plasma and 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	P01563

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 - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 µL/vial	1 vial	
Additional Diluent AT-00207 - 6 mL/bottle. Only for serum and plasma samples	1 bottle	
Sample Diluent PT 3-bfB1 - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants.	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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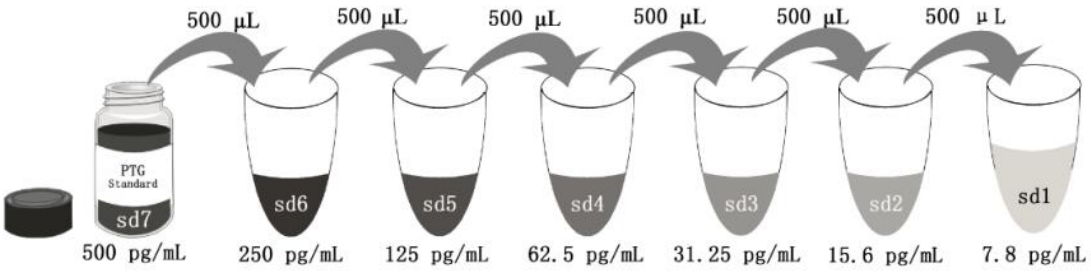
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-bfB1 is for protein standard, serum and plasma.
Sample Diluent PT 1-ef is for protein standard and cell culture supernatants samples.
Detection Diluent is for Detection antibody.
*Add **2mL** Sample Diluent PT 3-bfB1 or PT 1-ef in 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 3-bfB1 or 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 supernatants.

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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	Additional diluent (Only for serum and plasma sample test)	50 µL	0 min	Do not washDo not wash	Add additional diluent 50 µL per well then add standard and samples immediately
2	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
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.				

Assay Procedure Description

1. Prepare all reagents, samples and working standards as instructed.

(See Sandwich ELISA Kit Instruction Manual sections III. 1: Sample Preparation and 2: Reagent Preparation (including Standard Preparation)).

2. Take out the required number of microplate strips and place the microwells in the strip holder. In the meantime, return excess strips to the foil pouch containing the drying reagent pack, store at 4°C immediately. Microplate strips should be used as soon as possible.

3. Add 50 µL of each additional diluent to the appropriate wells (No need for incubation and wash).

(This step is only for serum and plasma sample test, not for cell culture supernatants).

4. Add 100 µL of standard or sample to the appropriate wells.

(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes).

5. Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C in a humid environment.

6. Wash wells:

i. Gently remove the cover seal.

ii. Discard the liquid from wells by aspirating or decanting.

iii. Remove any residual solution by tapping the plate a few times on fresh paper towels.

iv. Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Aviod getting and towel fibers in the wells or wells drying out completely.

7. Add 100 µL of 1X Detection Antibody, HRP-conjugated to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C in a humid environment. Repeat the washes in step 6.

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8. Signal development:

Add 100 µL of TMB substrate solution to each well. Incubate for 15 to 20 minutes at 37°C in the dark. A positive reaction will be indicated by the color blue.

(Longer incubation times are recommended in the event of the blue color appearing too pale)

9. Quenching color development:

Add 100 µL of Stop Soluton to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. The color will change from blue to yellow.

10. Read results:

Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm). The absorbance should be measurable immediately after adding Stop solution. DO NOT exceed 5 minutes.

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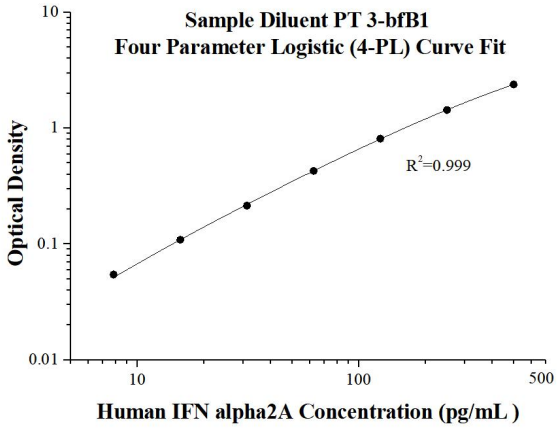
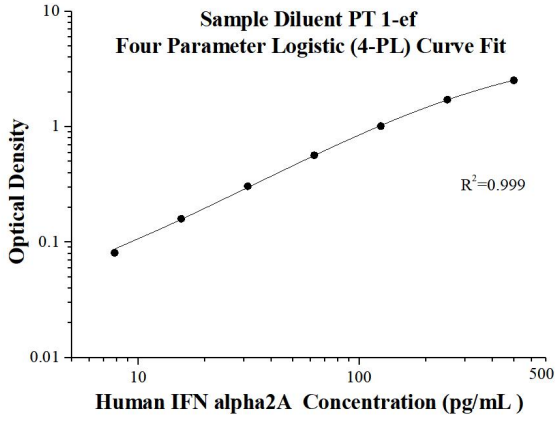
Example data

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	0.066 0.068	0.067	-
7.8	0.120 0.123	0.122	0.055
15.6	0.176 0.176	0.176	0.109
31.2	0.284 0.279	0.282	0.215
62.5	0.500 0.491	0.496	0.429
125	0.893 0.867	0.880	0.813
250	1.542 1.471	1.506	1.440
500	2.527 2.389	2.458	2.391



(pg/mL)	O.D	Average	Corrected
0	0.094 0.096	0.095	-
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 (%)
Human plasma	1:2	92	82-106
	1:4	105	95-124
Cell culture supernatants	1:2	95	85-102
	1:4	96	90-104

Sample Values

Serum -seven serum and eight plasma samples from healthy volunteers were evaluated for IFN alpha2A in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	%Detectable	Range (pg/mL)
Human serum and plasma samples (n=15)	66.5	40	29-185.5

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 alpha2A.

Condition	Day1 (pg/mL)
Unstimulated	-
Stimulated	159.7

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Sensitivity

The minimum detectable dose of human IFN alpha 2A is 1.17 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, serum and plasma samples were spiked with high concentrations of human IFN alpha 2A and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Cell culture supernatants were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma (PT 3-bfB1)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	89	100
	Range (%)	86-91	-
1:4	Average% of Expected	87	103
	Range (%)	80-91	102-104
1:8	Average% of Expected	98	106
	Range (%)	92-104	103-108
1:16	Average% of Expected	86	118
	Range (%)	76-95	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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