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Antibodies | ELISA kits | Proteins

AuthentiKine™

Human IFN-beta Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00195
Product Name	AuthentiKine™ Human IFN-beta Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3456
SwissProt	P01574

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 (100X) - 120 µL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4-eg - 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 (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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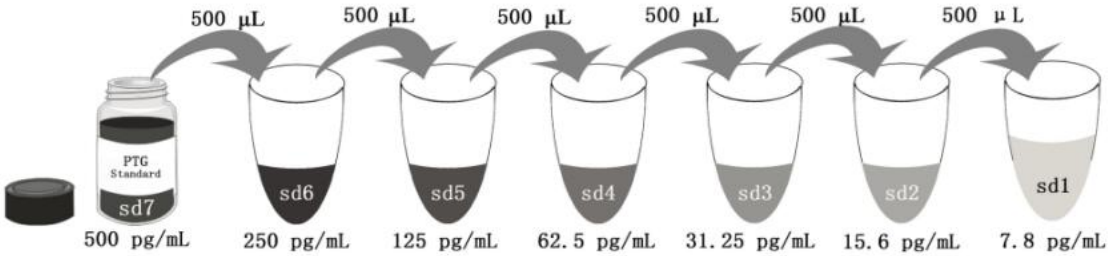
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4-eg is for protein standard, serum and plasma.
Sample Diluent PT 1-ef is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ef or PT 4-eg 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 or PT 4-eg	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00195 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IFN-beta ELISA kit is to be used to detect and quantify protein levels of endogenous IFN-beta. The assay recognizes human IFN-beta. An antibody specific for IFN-beta has been pre-coated onto the microwells. The IFN-beta protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IFN-beta is added to detect the captured IFN-beta protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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 beta (IFN-beta) is a cytokine that is naturally produced by the immune system in response to biological and chemical stimuli. It signals by binding to the heterodimeric type I IFN receptor composed of the IFNAR1 and IFNAR2 chains, and regulates the expression of a plethora of genes by means of the classical JAK/STAT and other pathways. Interferon beta (IFN-beta) has been shown in several clinical trials to have efficacy in multiple sclerosis. Interferon beta gene therapy is expected to become widely available for clinical use in cancer patients, and this new strategy might be extended to molecular targeting therapy, or used in combination with cell therapy or other therapies.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:4 dilution is recommended for serum and plasma, 1:8 or 1:16 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	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.				

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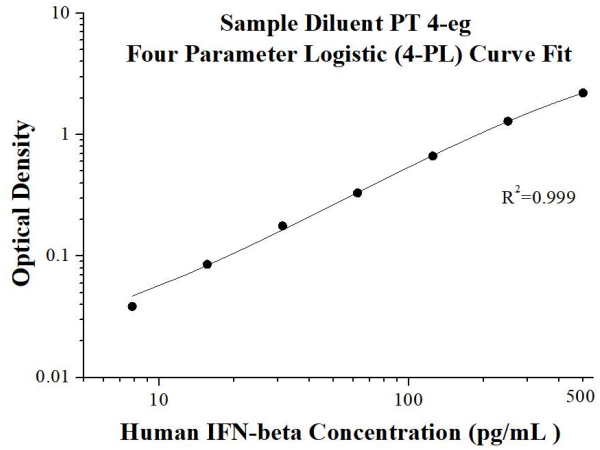
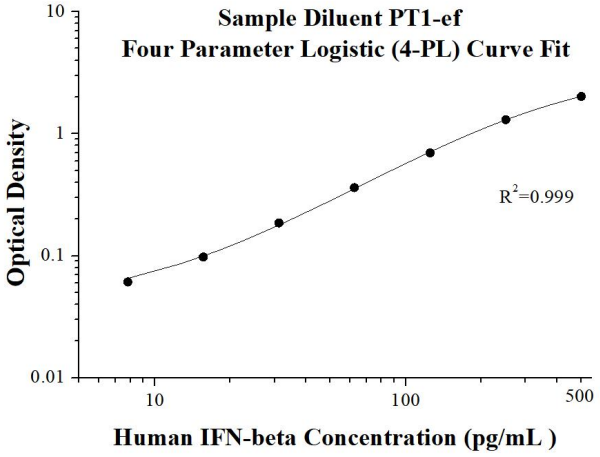
Example data

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	0.076 0.061	0.069	-
7.8	0.107 0.107	0.107	0.039
15.6	0.154 0.154	0.154	0.086
31.2	0.249 0.242	0.246	0.177
62.5	0.401 0.398	0.400	0.331
125	0.754 0.713	0.734	0.665
250	1.371 1.345	1.358	1.290
500	2.280 2.264	2.272	2.204



(pg/ml)	O.D	Average	Corrected
0	0.083 0.093	0.088	-
7.8	0.160 0.138	0.149	0.061
15.6	0.190 0.181	0.186	0.098
31.2	0.276 0.271	0.274	0.186
62.5	0.451 0.448	0.450	0.362
125	0.771 0.798	0.785	0.697
250	1.446 1.339	1.393	1.305
500	2.130 2.081	2.106	2.018

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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	244.5	5.2	2.1
2	20	61.6	1.7	2.8
3	20	14.9	1.3	8.8

Inter-assay Precision				
Sample	n	Mean (pg/ml)	SD	CV%
1	24	244.7	6.3	2.6
2	24	64.5	2.0	3.1
3	24	16.5	1.6	9.6

Recovery

The recovery of IFN-beta 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	1:4	82	72-91
	1:8	96	87-107
Cell culture supernatants	1:30	84	79-96
	1:60	83	78-91

Sample Values

Serum from healthy volunteers were evaluated for IFN-beta in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=8)	65.3	53.4-93.0

Cell Culture supernatants - A549 human lung carcinoma cells were cultured in Kaighn's Nutrient Mixture F-12 supplemented with 10% fetal bovine serum until nearly confluent. The cells were cultured unstimulated or stimulated with 10 ug/mL of poly I:C in the presence of 6ug/ml Lipofectamine 2000 (LF2K) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-beta.

Condition	(pg/mL)
Unstimulated for 1d	-
Stimulated for 1d	758.8

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Sensitivity

The minimum detectable dose of human IFN-beta is 0.76 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma and serum samples were initially diluted 1:2, the cell culture supernatants sample was initially diluted 1:4)

		Human serum (PT 4-eg)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	90	96
	Range (%)	81-108	92-103
1:8	Average% of Expected	107	95
	Range (%)	92-129	85-103
1:16	Average% of Expected		95
	Range (%)		83-110

Calibration

The NIBSC/WHO Human IFN-β Reference Reagent 00/572 (rDNA derived), which was intended as a potency standard, was evaluated in this kit. The dose response curve of this Reference Reagent parallels the Proteintech standard curve. To convert sample values obtained with the Authentikine Human IFN-beta ELISA kit to approximate NIBSC/WHO 00/572 values, use the equation below.

NIBSC/WHO (00/572) approximate value (IU/mL) = 0.64 x Authentikine Human IFN-beta value (pg/mL)

References

1. M Haji Abdolvahab. et al.(2016) Int Rev Cell Mol Biol. 326:343-72.
2. Revel M. et al. (2003) Pharmacol Ther. 100(1):49-62.
3. Jun Yoshida. et al. (2004) Cancer Sci. 95(11):858-65.

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