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AuthentiKine™

Human IL-27 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-27 concentrations in serum and plasma.

General Information

Catalogue Number	KE00182
Product Name	AuthentiKine™ Human IL-27 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	78.1-5000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	246778/10148
SwissProt	Q8NEV9 / Q14213

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 - 10000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4B1 - 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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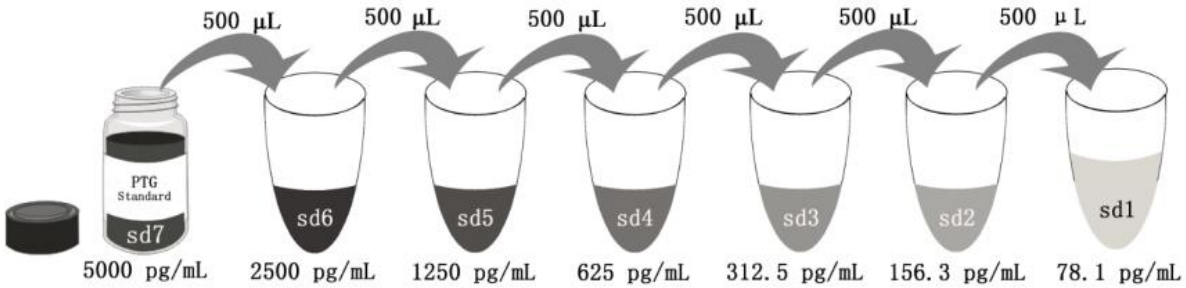
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4B1 is for protein standard and samples
Detection Diluent is for Detection antibody.
*Add **2 mL** Sample Diluent PT4B1 in protein standard. This reconstitution gives a stock solution of 5000 pg/mL.



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 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00182 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL27 ELISA kit is to be used to detect and quantify protein levels of endogenous IL27. The assay recognizes human IL27. An antibody specific for IL27 has been pre-coated onto the microwells. The IL27 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL27 is added to detect the captured IL27 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

IL27, a member of IL12/IL23 heterodimeric family of cytokines, has pleiotropic properties that can enhance or limit immune responses. IL27 is a heterodimeric cytokine composed of two subunits: IL27a and IL27b. IL27 acts on various cell types, including T cells, B cells, macrophages, dendritic cells, natural killer (NK) cells and non-hematopoietic cells. IL27 plays a critical role in the early regulation of T helper type 1 initiation, and enhances proliferation of naive CD4+T cells and naive B cells. It, however, also exerts anti-inflammatory functions by inhibiting the development of Th17 cells and inducing IL10 producing type 1 regulatory T cells. IL27 is a potentially promising cytokine for therapeutic approaches on various human diseases.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 dilution is recommended for serum or plasma.

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Safety Notes

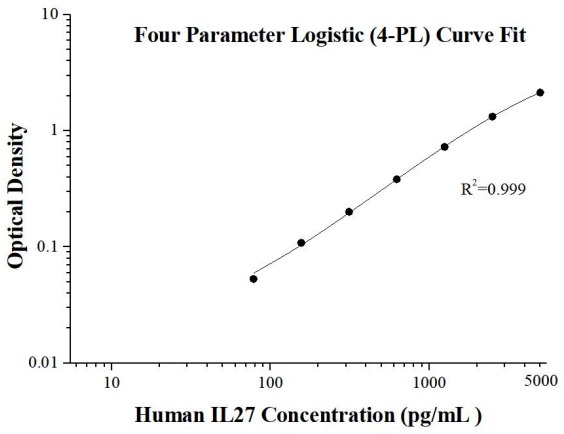
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	Detection Antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	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	0.040 0.041	0.041	-
78.1	0.091 0.096	0.094	0.053
156.2	0.150 0.148	0.149	0.109
312.5	0.240 0.242	0.241	0.201
625	0.422 0.425	0.424	0.383
1250	0.754 0.782	0.768	0.728
2500	1.365 1.370	1.368	1.327
5000	2.171 2.185	2.178	2.138

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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	2555.9	61.3	2.4
2	20	383.8	9.6	2.5
3	20	72.7	6.0	8.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	2667.3	79.7	3.0
2	24	411.7	10.2	2.5
3	24	94.9	7.5	7.9

Recovery

The recovery of IL27 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	101	80-114
	1:8	97	92-109

Sample Values

Samples from healthy volunteers were evaluated for IL27 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=20)	292.8	56.9-955.4

Sensitivity

The minimum detectable dose of human IL27 is 0.1 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.

		Human serum (PT 4B1)
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	107
	Range (%)	93-115
1:8	Average% of Expected	112
	Range (%)	97-127
1:16	Average% of Expected	108
	Range (%)	108-108

References

1. Hall AO. et al. (2012). Adv Immunol. 115:1-44.
2. Owaki T. et al. (2005). J Immunol. 175:2191-200.
3. Larousserie F. et al. (2006). J Immunol.176:5890-7.
4. Batten M. et al. (2006). Nat Immunol. 7:929-36.

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