

Human IL-27 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-27 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00089
Product Name	Human IL-27 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125 - 8000 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 - 8000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µ L/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 3-ec - 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	

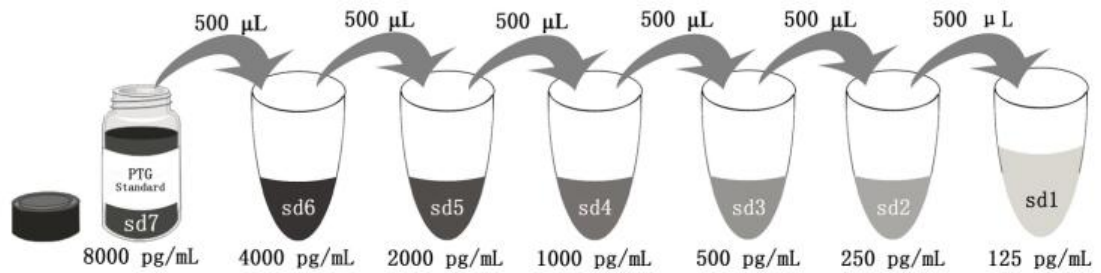
NB: Do not use the kit after the expiration date.

Sample Diluent PT 3-ec 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 Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 3-ec or PT 1-ef in 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 3-ec or PT 1-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00089 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The human IL27 ELISA kit is to be used to detect and quantify protein levels of endogenous human IL27. The assay recognizes human IL27. An antibody specific for human IL27 has been pre-coated onto the microwells. The human IL27 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human IL27 is added to detect the captured human IL27 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

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

The serum, plasma or cell culture supernatants samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

Safety Notes

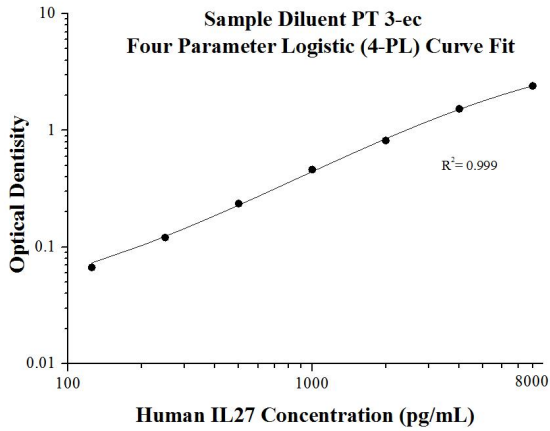
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

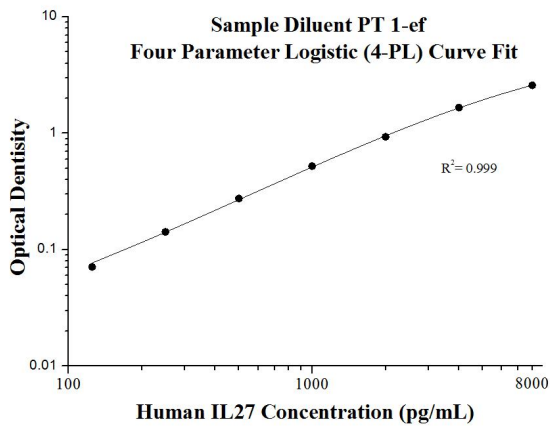
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μ L	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 μ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 μ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 μ L	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 μ L	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	0.074 0.077	0.076	-
125	0.142 0.144	0.143	0.067
250	0.191 0.203	0.197	0.121
500	0.318 0.306	0.312	0.236
1000	0.529 0.547	0.538	0.462
2000	0.924 0.866	0.895	0.819
4000	1.563 1.646	1.605	1.529
8000	2.453 2.502	2.478	2.402



(pg/mL)	O.D	Average	Corrected
0	0.071 0.072	0.072	-
125	0.14 0.146	0.143	0.071
250	0.207 0.221	0.214	0.142
500	0.361 0.332	0.347	0.275
1000	0.602 0.587	0.595	0.523
2000	1.01 0.997	1.004	0.932
4000	1.734 1.735	1.735	1.663
8000	2.597 2.723	2.660	2.588

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					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	1,767.4	79.1	4.5	1	24	1,959.9	80.9	4.1
2	20	3,341.6	106.2	3.2	2	24	3,633.0	206.3	5.7
3	20	6,796.1	527.0	7.8	3	24	6,731.5	532.8	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 serum	1:2	82	70-119
	1:4	89	72-120
Cell culture supernatants	1:2	101	96-117
	1:4	89	74-99

Sample Values

Twenty individual human serum samples were evaluated for the presence of human IL27 in this assay. All samples measured less than the lowest standard, 125 pg/mL.

Sensitivity

The minimum detectable dose of human IL27 is 11.0 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, three samples were spiked with high concentrations of human IL27 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum (Sample Diluent PT 3-ec)	Cell culture supernatants (Sample Diluent PT 1-ef)
1:2	Average% of Expected	102	102
	Range (%)	93-118	91-116
1:4	Average% of Expected	102	106
	Range (%)	84-117	104-109
1:8	Average% of Expected	101	102
	Range (%)	93-109	93-108
1:16	Average% of Expected	98	94
	Range (%)	85-107	82-102

References

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