

Human IL-23 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IL-23 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00088
Product Name	Human IL-23 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	51561
SwissProt	Q9NPF7

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, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 5-ec - 30 mL/bottle. For serum and plasma samples	1 bottle	
Sample Diluent PT 5-of - 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	

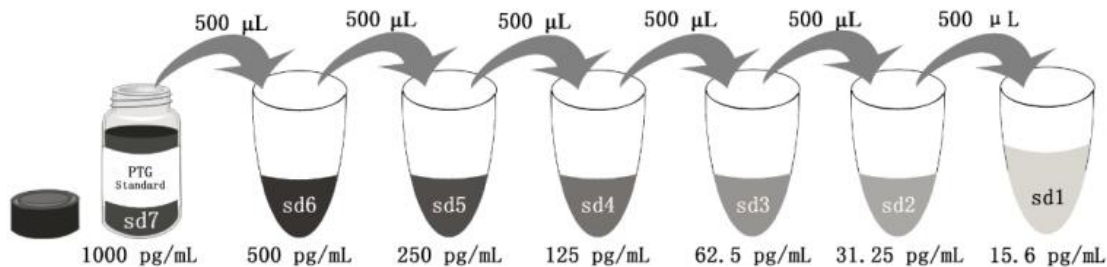
NB: Do not use the kit after the expiration date.

Sample Diluent PT 5-ec is for protein standard, serum and plasma samples.

Sample Diluent PT 5-of is for protein standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 5-ec or PT 5-of in protein standard. This reconstitution gives a stock solution of 1000 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 5-ec or PT 5-of	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00088 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL23 ELISA kit is to be used to detect and quantify protein levels of endogenous IL23. The assay recognizes human IL23. An antibody specific for IL23 has been pre-coated onto the microwells. The IL23 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL23 is added to detect the captured IL23 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

Interleukin 23 (IL23) is a member of the IL12 cytokine family and composed of two subunits, IL12p40 and IL23p19. It is produced by antigen presenting cells and has been shown to promote the production and survival of a distinct lineage of T-cells called TH17 cells. A functional receptor for IL23 (the IL23 receptor) has been identified and is composed of IL12R β 1 and IL23R. IL23 is expressed chiefly by the macrophages and DCs. The IL-23R is found on memory T cells, NKT cells, macrophages, DCs, and naive T cells upon activation by TGF- β and IL6. The main biological effects of IL23 identified initially consist of stimulation of antigen presentation by DCs, T cell differentiation to Th17 cells, and production of interferon- γ (IFN- γ). IL23 acts also as an end-stage effector cytokine through direct action on macrophages.

Sample Preparation

The serum or plasma 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

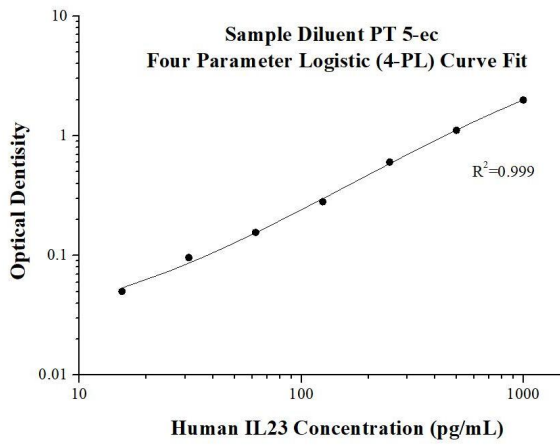
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

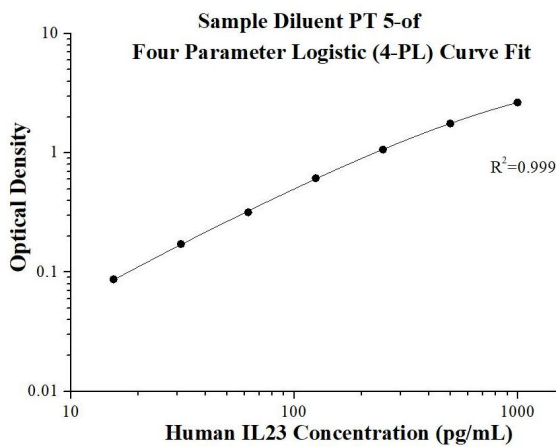
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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.				

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	0.109 0.107	0.108	-
15.6	0.153 0.163	0.158	0.05
31.25	0.205 0.203	0.204	0.096
62.5	0.244 0.284	0.264	0.156
125	0.443 0.335	0.389	0.281
250	0.704 0.719	0.712	0.604
500	1.2 1.242	1.221	1.113
1000	2.049 2.155	2.102	1.994



(pg/mL)	O.D	Average	Corrected
0	0.039 0.038	0.0385	-
15.6	0.126 0.125	0.1255	0.087
31.25	0.207 0.214	0.2105	0.172
62.5	0.336 0.376	0.356	0.318
125	0.625 0.68	0.6525	0.614
250	1.076 1.135	1.1055	1.067
500	1.818 1.796	1.807	1.769
1000	2.7 2.681	2.6905	2.652

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	775.2	58.7	7.6
2	20	180.3	15.8	8.8
3	20	20.0	1.2	5.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	770.1	61.5	8.0
2	24	166.4	15.3	9.2
3	24	13.7	1.2	9.0

Recovery

The recovery of IL23 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	82-116
	1:4	97	83-117
Cell culture supernatants	1:2	88	82-92
	1:4	104	92-120

Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL23 in this assay. All samples measured less than the lowest standard, 15.6 pg/mL. No medical histories were available for the donors used in this study.

Sensitivity

The minimum detectable dose of human IL23 is 3.3 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 IL23 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma (Sample Diuent PT 5-ec)	Cell culture supernatants (Sample Diuent PT 5-of)
1:2	Average% of Expected	82	93
	Range (%)	81-82	82-103
1:4	Average% of Expected	78	97
	Range (%)	77-79	80-114
1:8	Average% of Expected	83	115
	Range (%)	82-84	82-116
1:16	Average% of Expected	89	113
	Range (%)	87-91	80-115

References

1. Kleinschek MA. et al.(2006). J Immunol. 176: 1098-106.
2. Langrish CL. et al.(2005). J Exp Med. 201:233-40.
3. Parham C. et al.(2002). Journal of Immunology. 168 (11): 5699-708.
4. Cua DJ. et al. (2003). Nature.421: 744-8.