

Human IL-13 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-13 in serum, plasma, and cell culture supernatants.

General Information

Catalogue Number	KE00020
Product Name	Human IL-13 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	62.5-4000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3596
SwissProt	P35225

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-ec - 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	

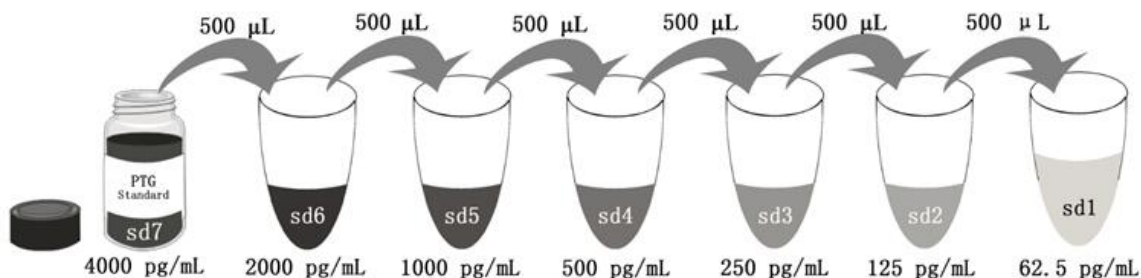
NB: Do not use the kit after the expiration date.

Sample Diluent PT 3-ec is for protein standard, serum and plasma.

Sample Diluent PT 1-ec is for protein standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 3-ec or PT 1-ec in standard. This reconstitution gives a stock solution of 4000 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 PT1-ec	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00020 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-13 ELISA kit is to be used to detect and quantify protein levels of endogenous IL-13. The assay recognizes human IL-13. An antibody specific for IL-13 has been pre-coated onto the microwells. The IL-13 protein in samples is captured by the coated antibody after incubation.

Following extensive washing, another antibody of biotinylated specific for IL-13 is added to detect the captured IL-13 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 13 (IL-13) is an immunoregulatory cytokine produced primarily by activated Th2 cells. This cytokine is involved in several stages of B-cell maturation and differentiation. IL-13 up-regulates CD23 and MHC class II expression, and promotes IgE isotype switching of B cells. IL-13 inhibits the production of a series of cytokines like IL-1, IL-6, TNF-alpha, and IL-8 by activated human monocytes. IL-13 induces IFN-gamma production by NK cells. IL-13 is thought to be important cytokine in the pathogenesis of asthma, and more recently has been shown to play a pivotal role in a number of fibrotic diseases including hepatic and pulmonary fibrosis, and nodular sclerosing Hodgkin's disease.

Sample Preparation

The 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

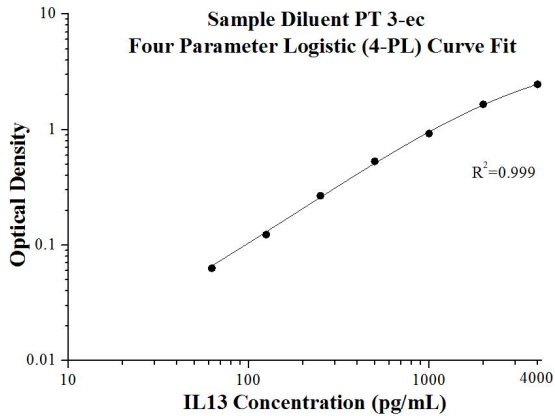
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

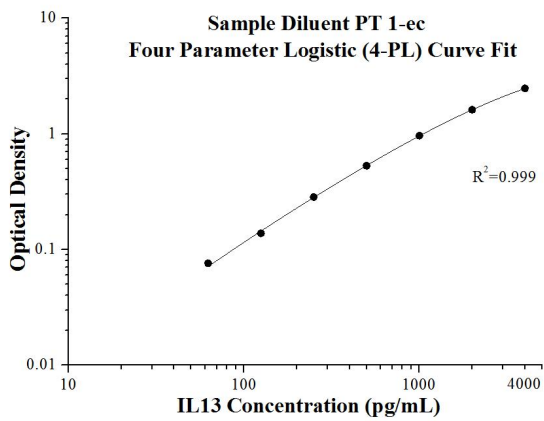
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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	0.024 0.025	0.025	-
62.5	0.095 0.081	0.088	0.063
125	0.154 0.143	0.149	0.124
250	0.304 0.281	0.293	0.268
500	0.575 0.538	0.557	0.532
1000	1.008 0.887	0.948	0.923
2000	1.735 1.62	1.678	1.653
4000	2.529 2.426	2.489	2.464



(pg/mL)	O.D	Average	Corrected
0	0.019 0.019	0.019	-
62.5	0.089 0.101	0.095	0.076
125	0.154 0.159	0.157	0.138
250	0.315 0.291	0.303	0.284
500	0.559 0.536	0.548	0.529
1000	1.004 0.962	0.983	0.964
2000	1.633 1.624	1.629	1.61
4000	2.501 2.472	2.487	2.468

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	385.0	23.4	6.1	1	24	374.7	23.2	6.2
2	20	848.6	51.8	6.1	2	24	840.6	68	8.1
3	20	3,209.4	127.8	4.0	3	24	3,204.9	315.4	9.8

Recovery

The recovery of IL-13 spiked to three different levels in four samples throughout the range of the assay in cell culture supernatants were evaluated. (The serum and plasma samples were initially diluted 1:2)

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	86	81-94
	1:4	90	81-95
Cell culture supernatants	1:2	89	81-96
	1:4	79	73-81

Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL-13 in this assay. All samples measured less than the lowest standard, 62.5 pg/mL. No medical histories were available for the donors used in this study.

Sensitivity

The minimum detectable dose of human IL-13 is 1.0 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 IL-13 in matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human Plasma (Sample Diluent PT 3-ec)	Cell culture supernatants (Sample Diluent PT 1-ec)
1:2	Average% of Expected	99	93
	Range (%)	97-102	90-97
1:4	Average% of Expected	104	101
	Range (%)	99-109	97-106
1:8	Average% of Expected	105	93
	Range (%)	105-106	88-97
1:16	Average% of Expected	106	91
	Range (%)	100-111	88-93

References

1. Wynn TA. et al.(2003). Annu Rev Immunol. 21: 425-56.
2. Izuhara K. et al. (2006). Curr Med Chem. 13: 2291-8.
3. Corren J. et al. (2015). Curr Allergy Asthma Rep. 13: 415-20.
4. O'Reilly S. et al. (2013). Biofactors. 39: 593-6.