Human IL-31 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-31 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00086
Product Name	Human IL-31 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25 - 2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	386653
SwissProt	Q6EBC2

Kit Components & Storage
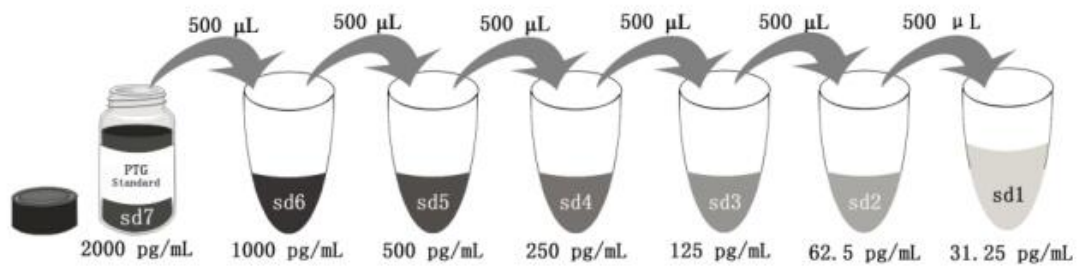
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months.
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-af - 30 mL/bottle	1 bottle	**Opened Kit:** All reagents stored at 2-8°C for 7 days.
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	**Please use a new standard for each assay.**
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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-af	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00086 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Human IL31 ELISA kit is to be used to detect and quantify protein levels of endogenous human IL31. The assay recognizes human IL31. An antibody specific for human IL31 has been pre-coated onto the microwells. The human IL31 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human IL31 is added to detect the captured human IL31 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-31 (IL-31) is a newly discovered four-helix bundle cytokine and expressed by several types of cells under both normal and disease state, such as activated CD4+T cells, mast cells, monocytes, macrophages, immature and mature monocyte-derived dendritic cells and so on. IL-31 signals through a heterodimeric receptor complex consisting of the IL31 receptor alpha (IL31R) and the oncostatin M receptor (OSMR). IL-31 plays a predominant function in the mediation of inflammatory and lymphoma-associated itch. Increased IL-31 expression has been detected in inflamed tissues from patients with atopic dermatitis, bowel diseases, allergic asthma and rhinitis.

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.
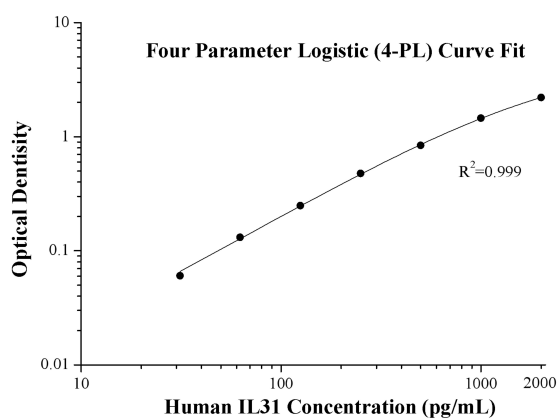
Safety Notes

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 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.

Four Parameter Logistic (4-PL) Curve Fit
$R^2=0.999$
Optical Dentisity
Human IL31 Concentration (pg/mL)

(pg/mL)	O.D	Average	Corrected
0	0.066 0.066	0.066	-
31.25	0.125 0.128	0.127	0.061
62.5	0.192 0.203	0.198	0.132
125	0.302 0.327	0.315	0.249
250	0.546 0.538	0.542	0.476
500	0.906 0.905	0.906	0.840
1000	1.529 1.509	1.517	1.453
2000	2.314 2.23	2.272	2.206

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	41.0	1.7	4.2
2	20	431.6	10.7	2.5
3	20	1,731.5	52.7	3.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	37.4	3.3	8.9
2	24	415.9	15.1	3.6
3	24	1,726.2	74.8	4.3

Recovery

The recovery of IL31 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	86	74-117
	1:4	96	80-117
Cell culture supernatants	1:2	92	84-113
	1:4	90	83-97

Sample Values

Sixteen individual human serum samples were evaluated for the presence of human IL31 in this assay.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=16)	861.1	69. 1- 3,057.8

Sensitivity

The minimum detectable dose of human IL31 is 7.4 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 IL31 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	90	100
	Range (%)	89-91	90-109
1:4	Average% of Expected	98	98
	Range (%)	90-106	92-106
1:8	Average% of Expected	109	101
	Range (%)	101-120	92-104
1:16	Average% of Expected	114	102
	Range (%)	108-121	96-107

References

1. Zhang Q. et al. (2008) Cytokine Growth Factor Rev. 19:347-56.
2. Dillon SR. et al.(2004) Nat Immunol. 5:752-60.
3. Sonkoly E. et al. (2006) J Allergy Clin Immunol. 117: 411-417.
4. Bilsborough J. et al. (2006) J Allergy Clin Immunol. 117: 418-425.
5. Raap U.et al. (2008) J Allergy Clin Immunol. 122: 421-423.