Human IL-28B Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-28B in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00014
Product Name	Human IL-28B Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	282617
SwissProt	Q8IZI9

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 - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ec - 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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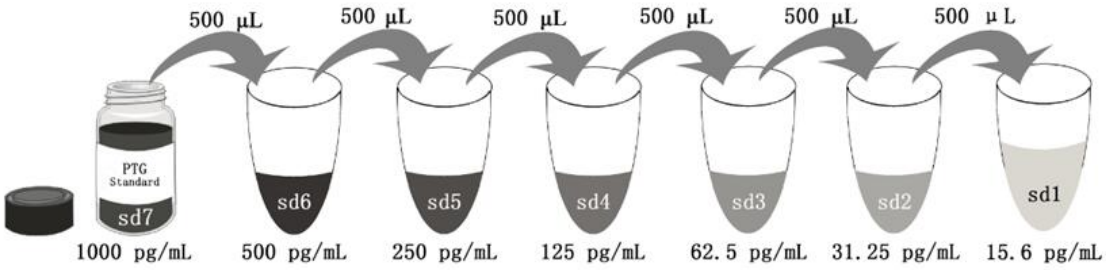
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ec is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ec in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



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 1-ec	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00014 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL28B ELISA kit is to be used to detect and quantify protein levels of endogenous IL28B. The assay recognizes human IL28B. An antibody specific for IL28B has been pre-coated onto the microwells. The IL28B protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL28B is added to detect the captured IL28B protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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

IL28B, also named as IFNL3, is a cytokine distantly related to type I interferons and the IL-10 family. IL28B, interleukin 28A (IL28A), and interleukin 29 (IL29) are three closely related cytokine genes that form a cytokine gene cluster on a chromosomal region mapped to 19q13. Expression of the cytokines encoded by the three genes can be induced by viral infection. All three cytokines have been shown to interact with a heterodimeric class II cytokine receptor that consists of interleukin 10 receptor, beta (IL10RB) and interleukin 28 receptor, alpha (IL28RA).

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.

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Safety Notes

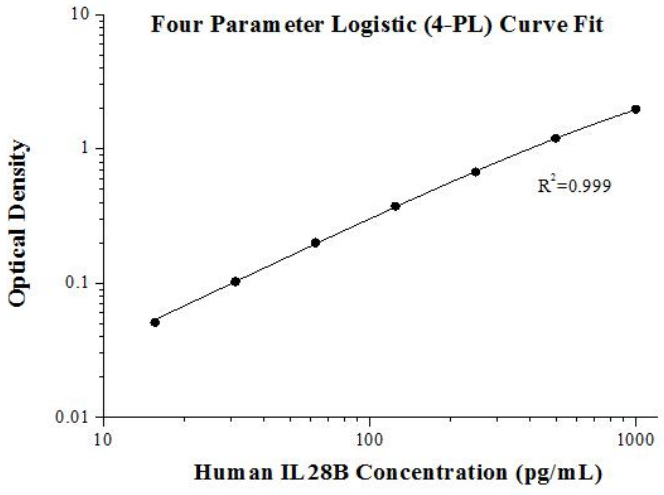
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	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	0.07 0.072	0.071	—
15.63	0.125 0.119	0.122	0.051
31.25	0.176 0.171	0.1735	0.1025
62.5	0.272 0.269	0.2705	0.1995
125	0.452 0.439	0.4455	0.3745
250	0.752 0.732	0.742	0.671
500	1.308 1.239	1.2735	1.2025
1000	2.03 2.032	2.031	1.96

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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	577.0	23.2	4.0
2	20	189.4	7.7	4.0
3	20	45.8	2.3	4.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	567.3	28.7	5.1
2	24	176.1	6.3	3.6
3	24	39.8	2.3	5.8

Recovery

The recovery of IL-28B 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	102	97-109
	1:8	105	86-122
Cell culture supernatants	1:2	97	85-113
	1:4	96	91-99

Sensitivity

The minimum detectable dose of human IL-28B is 0.8 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, three samples were spiked with high concentrations of IL-28B in various matrices and diluted with the appropriate **Sample Diluent PT 1-ec** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	106	100
	Range (%)	101-109	80-112
1:4	Average% of Expected	109	98
	Range (%)	107-111	83-108
1:8	Average% of Expected	110	100
	Range (%)	107-114	86-118
1:16	Average% of Expected	108	102
	Range (%)	101-117	88-126

References

1. Sheppard P. et al. (2003). Nat Immunol. 4: 63-8.
2. provided by RefSeq, Jul 2008

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