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Antibodies | ELISA kits | Proteins

AuthentiKine™

Human IL-28B Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00235
Product Name	AuthentiKine™ 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, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3 - 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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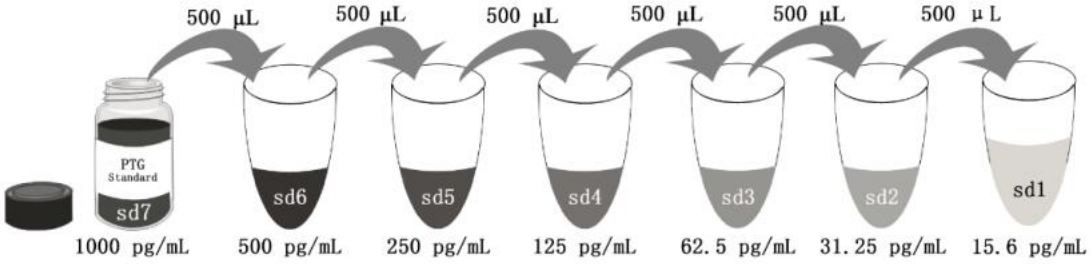
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3 is for protein standard and samples.
Detection Diluent is for Detection antibody.
*Add **2 mL** Sample Diluent PT 3 in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



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 3	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00235 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-28B ELISA kit is to be used to detect and quantify protein levels of endogenous IL-28B. The assay recognizes human IL-28B. An antibody specific for IL-28B has been pre-coated onto the microwells. The IL-28B protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL-28B is added to detect the captured IL-28B protein. For signal development, 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

IL-28B, also named as IFNL3, is a cytokine distantly related to type I interferons and the IL-10 family. IL-28B, interleukin 28A (IL-28A), and interleukin 29 (IL-29) 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 (IL-10RB) and interleukin 28 receptor, alpha (IL-28RA). IL-28B was identified as a key regulator of B- and T-cell vaccine responses against influenza.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 or 1:4 dilution is recommended for cell culture supernatants.

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Safety Notes

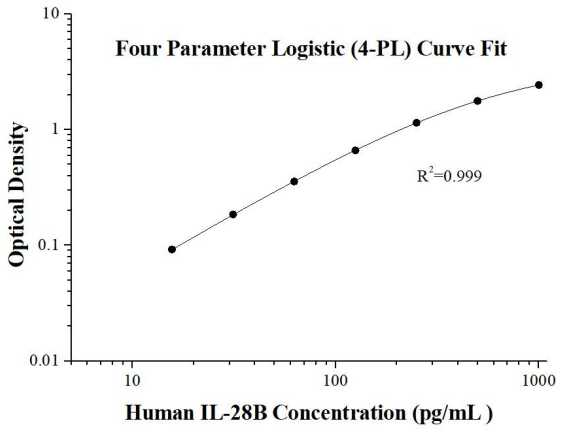
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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 Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	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	0.014 0.013	0.0135	-
15.6	0.104 0.107	0.1055	0.092
31.25	0.194 0.203	0.1985	0.185
62.5	0.366 0.374	0.37	0.3565
125	0.672 0.68	0.676	0.6625
250	1.175 1.15	1.1625	1.149
500	1.761 1.811	1.786	1.7725
1000	2.452 2.439	2.4455	2.432

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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	423.9	7.1	1.7
2	20	103.5	2.6	2.6
3	20	22.3	0.4	1.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	427.6	9.0	2.1
2	24	101.5	1.1	1.1
3	24	22.8	0.5	2.2

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	1:2	100	95-108
	1:4	102	97-112
Cell culture supernatants	1:4	114	103-123
	1:8	108	98-116
Human urine	1:2	94	90-98
	1:4	98	94-102

Sample Values

Serum - Sixteen serum samples from healthy volunteers were evaluated for human IL-28B 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.

Urine -Five urine samples from healthy volunteers were evaluated for human IL-28B 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.

Cell Culture supernatants - A549 human lung carcinoma cells were cultured in Kaighn's Nutrient Mixture F-12 supplemented with 10% fetal bovine serum until nearly confluent. The cells were cultured unstimulated or stimulated with 10 ug/mL of poly I:C in the presence of 10ug/mL Lipofectamine 2000 (LF2K) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-28B.

Condition	(pg/mL)
Unstimulated for 1d	-
Stimulated for 1d	3,608.9

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Sensitivity

The minimum detectable dose of human IL-28B is 2.1 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, serum,plasma and urine samples were spiked with high concentrations of human IL-28B and diluted with the appropriate Sample Diluent **PT 3** to produce samples with values within the dynamic range of the assay. Cell culture supernatants were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.(The cell culture supernatants were initially diluted 1:2)

		Human plasma	Cell culture supernatants	Urine
1:2	Average% of Expected	100	100	92
	Range (%)	96-101	-	85-97
1:4	Average% of Expected	106	95	97
	Range (%)	100-109	94-97	95-98
1:8	Average% of Expected	106	97	102
	Range (%)	98-109	95-100	101-103
1:16	Average% of Expected	109	96	109
	Range (%)	102-112	87-100	106-113

References

1. Sheppard P. et al. (2003). Nat Immunol. 4: 63-8.
2. Katrin Witte. et al. (2010) Cytokine Growth Factor Rev. 21(4):237-51.
3. Christabel Kelly. et al. (2011) Gut. 60(9):1284-93.
4. Adrian Egli.et al. (2014) PLoS Pathog.10(12):e1004556.

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