

Human IL-28A Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00011
Product Name	Human IL-28A Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	282616
SwissProt	Q45KQ8

Kit Components & Storage

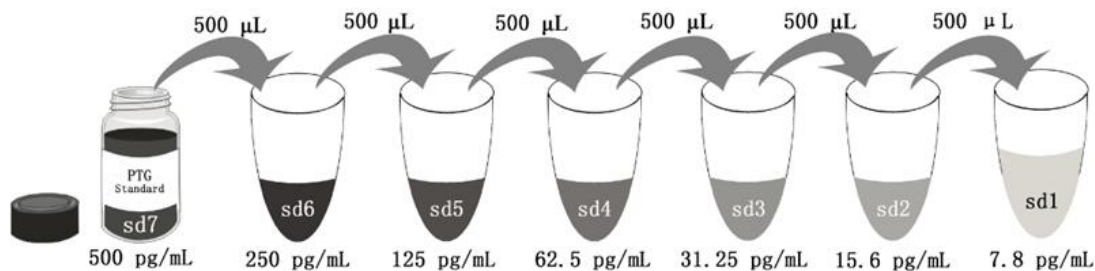
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 1-ef - 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	

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

Sample Diluent PT 1-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

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

Product Description

KE00011 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL28A ELISA kit is to be used to detect and quantify protein levels of endogenous IL28A. The assay recognizes human IL28A. An antibody specific for IL28A has been pre-coated onto the microwells. The IL28A protein in samples is captured by the coated antibody after incubation.

Following extensive washing, another antibody specific for IL28A is added to detect the captured IL28A 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

IL28A, also known as IFNL2, is a cytokine distantly related to type I interferons and the IL-10 family. IL-28A, IL-28B and IL-29 are three closely related cytokines classified as type III IFNs, which share many of the biological effects of type I IFNs but may have fewer side effects due to a more selective receptor distribution. 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). IL28A is believed to play a significant role in the antiviral immune defense in the intestinal epithelium.

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

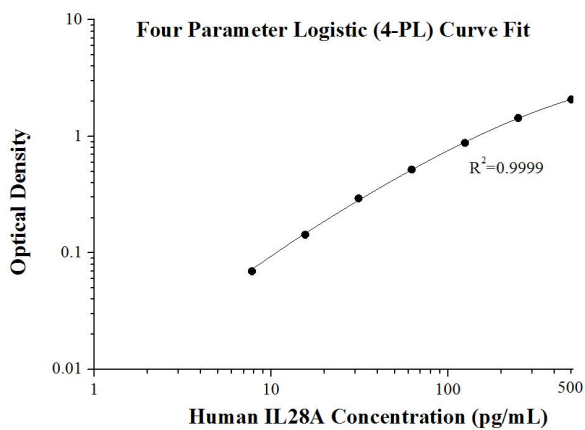
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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	0.032 0.035	0.0335	—
7.8	0.107 0.099	0.103	0.0695
15.63	0.173 0.18	0.1765	0.143
31.25	0.321 0.333	0.327	0.2935
62.5	0.557 0.548	0.5525	0.519
125	0.917 0.906	0.9115	0.878
250	1.495 1.451	1.473	1.4395
500	2.112 2.107	2.1095	2.076

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	357.5	34.2	9.6
2	20	89.6	5.1	5.7
3	20	23.2	0.9	4.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	375.8	22.8	6.1
2	24	95.1	4.4	4.6
3	24	23.1	1.0	4.4

Recovery

The recovery of IL-28A 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	95	87-102
	1:4	94	87-99
Cell culture supernatants	1:2	102	92-109
	1:4	100	88-107
Urine	1:2	96	89-101
	1:4	104	100-109

Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL-28A in this assay. All samples measured less than the lowest standard, 7.8 pg/mL. No medical histories were available for the donors used in this study.

Sensitivity

The minimum detectable dose of human IL-28A 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-28A in various matrices and diluted with the appropriate **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma	Cell culture supernatants	Urine
1:2	Average% of Expected	94	98	95
	Range (%)	84-107	95-101	94-97
1:4	Average% of Expected	103	97	99
	Range (%)	89-111	95-98	96-101
1:8	Average% of Expected	107	101	101
	Range (%)	99-115	90-111	100-101
1:16	Average% of Expected	104	100	100
	Range (%)	93-111	94-108	97-102

References

1. Donnelly RP. et al. (2010). J Interferon Cytokine Res. 30: 55-64.
2. Sheppard P. et al. (2003). Nat Immunol. 4: 63-8.
3. Li MC. et al. (2006). Acta Pharmacol Sin. 27: 453-9.