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AuthentiKine™

Human IL-28A Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00237
Product Name	AuthentiKine™ Human IL-28A Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 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 - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 µL/vial	1 vial	
Additional Diluent AT-00237 - 6 mL/bottle. Only for serum and plasma samples	1 bottle	
Sample Diluent PT 4B2 - 30 mL/bottle. For human serum and plasma	1 bottle	
Sample Diluent PT 3 - 30 mL/bottle. For cell culture supernatants	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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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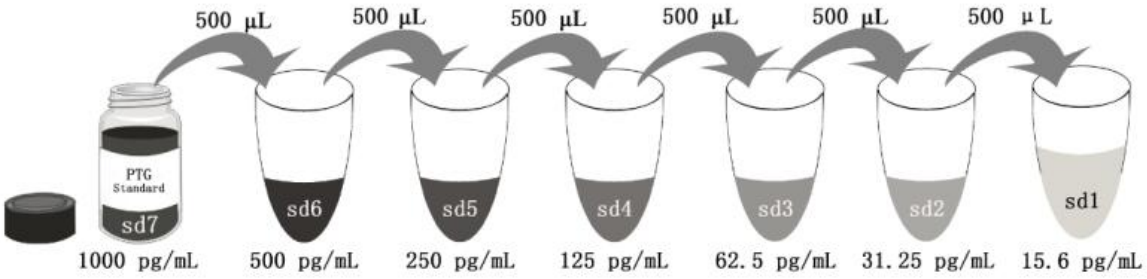
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4B2 is for protein standard, serum and plasma samples.
Sample Diluent PT 3 is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody .
*Add **2 mL** Sample Diluent PT 4B2 or PT 3 in protein standard. 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 4B2 or 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

KE00237 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-28A ELISA kit is to be used to detect and quantify protein levels of endogenous IL-28A. The assay recognizes human IL-28A and IL-28B. An antibody specific for IL-28A has been pre-coated onto the microwells. The IL-28A protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL-28A is added to detect the captured IL-28A 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

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

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	Additional diluent (Only for serum and plasma sample test)	50 µL	0 min	Do not wash	Cover Wells incubate at 37°C
2	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
3	Diluent Detection antibody, HRP-conjugated 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.				

Data Analysis

1. Prepare all reagents, samples and working standards as instructed.
(See Sandwich ELISA Kit Instruction Manual sections III. 1: Sample Preparation and 2: Reagent Preparation (including Standard Preparation)).
2. Take out the required number of microplate strips and place the microwells in the strip holder. In the meantime, return excess strips to the foil pouch containing the drying reagent pack, store at 4°C immediately. Microplate strips should be used as soon as possible.
3. Add 50 µL of each additional diluent to the appropriate wells (No need for incubation and wash).
(This step is only for serum and plasma sample test, not for cell culture supernatants).
4. Add 100 µL of each standard and sample to the appropriate wells.
(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes).
5. Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C in a humid environment.
6. Wash wells:
i. Gently remove the cover seal.
ii. Discard the liquid from wells by aspirating or decanting.
iii. Remove any residual solution by tapping the plate a few times on fresh paper towels.
iv. Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Aviod getting and towel fibers in the wells or wells drying out completely.
7. Add 100 µL of 1X Detection Antibody, HRP-conjugated to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C in a humid environment. Repeat the washes in step 6.
8. Signal development:
Add 100 µL of TMB substrate solution to each well. Incubate for 15 to 20 minutes at 37°C in the dark. A positive reaction will be indicated by the color blue.
(Longer incubation times are recommended in the event of the blue color appearing too pale).
9. Quenching color development:
Add 100 µL of Stop Soluton to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. The color will change from blue to yellow.
10. Read results:
Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm). The absorbance should be measurable immediately after adding Stop solution. DO NOT exceed 5 minutes.

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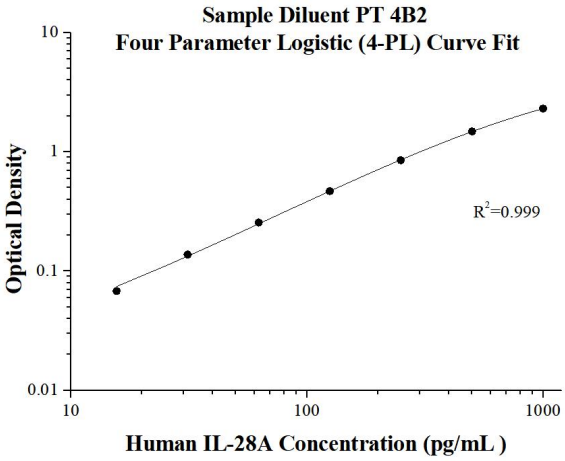
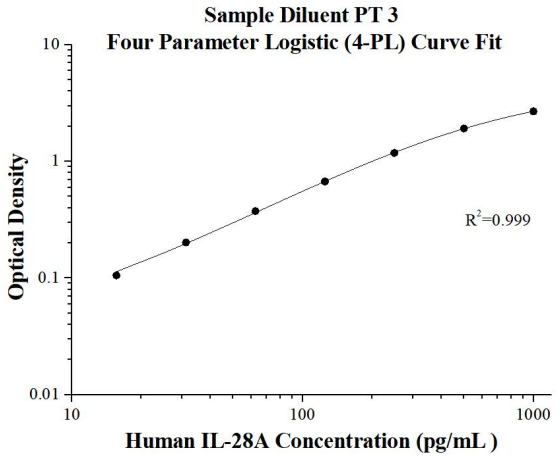
Example data

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	0.036 0.036	0.036	-
15.6	0.105 0.103	0.104	0.068
31.25	0.177 0.17	0.1735	0.1375
62.5	0.307 0.276	0.2915	0.2555
125	0.518 0.491	0.5045	0.4685
250	0.888 0.888	0.888	0.852
500	1.53 1.511	1.5205	1.4845
1000	2.355 2.334	2.3445	2.3085



(pg/mL)	O.D	Average	Corrected
0	0.046 0.045	0.0455	-
15.6	0.151 0.151	0.151	0.1055
31.25	0.246 0.249	0.2475	0.202
62.5	0.416 0.425	0.4205	0.375
125	0.702 0.734	0.718	0.6725
250	1.207 1.252	1.2295	1.184
500	1.934 1.998	1.966	1.9205
1000	2.706 2.759	2.7325	2.687

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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	440.6	11.5	2.6
2	20	102.2	3.1	3.1
3	20	23.8	0.4	1.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	428.9	10.7	2.5
2	24	97.7	1.6	1.6
3	24	24.1	0.9	3.9

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		Range (%)	Average% of Expected
Human plasma	1:2	76-87	80
	1:4	72-89	80
Cell culture supernatants	1:32	88-101	96
	1:64	91-103	98

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Sample Values

Serum - eight serum and eight plasma samples from healthy volunteers were evaluated for human IL-28A in this assay. serum samples measured less than the lowest standard, 15.6 pg/mL. two plasma samples measured 20.88 pg/ml and 185.10 pg/ml respectively,other six plasma samples measured less than the lowest standard, 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 5 ug/mL Lipofectamine 2000 (LF2K) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-28A.

Condition	(pg/mL)
Unstimulated for 1d	-
Stimulated for 1d	3,774.2

HUVEC cells were stimulated by adding human IFN-alpha at 100 ng/ml for 20 hours, followed by the addition of 30 μg/mL Poly(I:C) for 3 hours in Endothelial Cell Medium(ECM) . liquots of the cell culture supernates were removed and assayed for levels of human IL-28A.

Condition	(pg/mL)
Unstimulated	-
Stimulated	70.0

Sensitivity

The minimum detectable dose of human IL-28A is 0.12 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, serum and plasma samples were spiked with high concentrations of human IL-28A and diluted with the appropriate Sample Diluent 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.

Sample Type		Range (%)	Average% of Expected
Human plasma (PT 4B2)	1:2	77-87	81
	1:4	81-90	85
	1:8	90-99	94
	1:16	106-108	107
Cell culture supernatants (PT 3)	1:8	-	100
	1:16	94-100	98
	1:32	90-104	98
	1:64	88-112	99

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.

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