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AuthentiKine™

Human IL-4 Sandwich ELISA Kit Datasheet

For the quantitative detection of human IL-4 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00232
Product Name	AuthentiKine™ Human IL-4 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3565
SwissProt	P05112

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, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Additional Diluent AT-00232 - 6 mL/bottle. Only for serum and plasma samples	1 bottle	
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	

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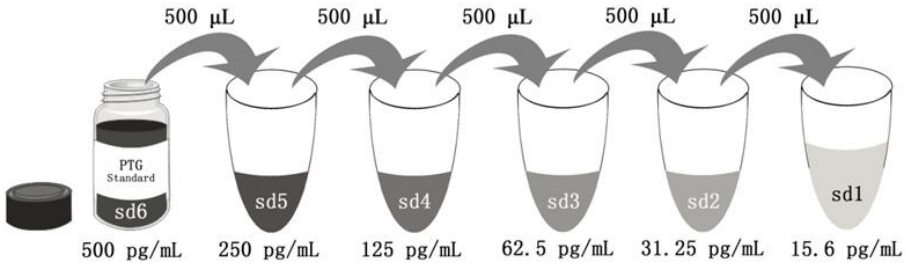
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody,HRP-conjugated.
*Add 2 **mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00232 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-4 ELISA kit is to be used to detect and quantify protein levels of endogenous IL-4. The assay recognizes human IL-4. An antibody specific for IL-4 has been pre-coated onto the microwells. The IL-4 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL-4 is added to detect the captured IL-4 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

Interleukin-4 (IL-4), a member of the α-helical cytokine family, is produced by activated CD4 + T cells, basophils, and mast cells. It promotes the proliferation and differentiation of antigen presenting cells. IL-4 also plays a pivotal role in antibody isotype switching and stimulates the production of IgE. This cytokine has been applied in the treatment of autoimmune disorder like multiple myeloma, cancer, psoriasis, and arthritis. IL-4 has also been extensively applied to inhibit detrimental effect of Th1. It may promote the growth of epithelial tumors by mediating increased proliferation and survival.

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 for serum or plasma samples , no diluted or 1:2 is suggested for cell culture supernatants 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	Additional diluent (Only for serum and plasma sample test)	50 uL	0min	Do not wash	Add additional diluent 50 µL per well then add standard and samples immediately
2	Standard and Samples	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent Detection antibody, HRP-conjugated Solution	100 µL	60 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.				

Assay Procedure Description

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℃ 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 1 hours at 37℃ 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 1 hour at 37℃ in a humid environment. Repeat the washes in step 5.

8. Signal development:

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Add 100 µL of TMB substrate solution to each well. Incubate for 15 to 20 minutes at 37℃ 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:

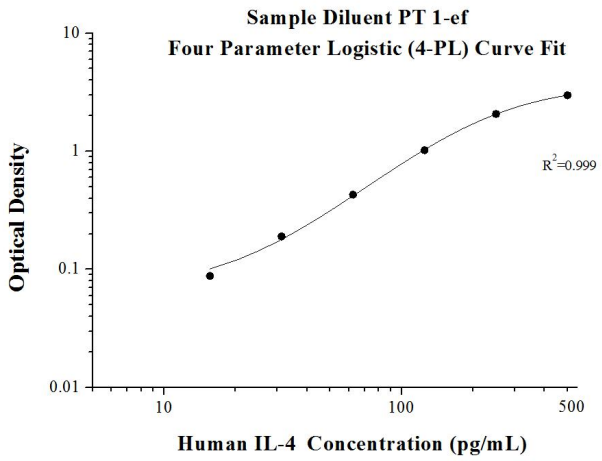
Add 100 µL of Stop Soluton to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. The color will change from blue to yellow.

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.

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	0.028 0.031	0.029	-
15.6	0.073 0.078	0.076	0.046
31.25	0.127 0.14	0.134	0.104
62.5	0.289 0.287	0.288	0.259
125	0.714 0.751	0.733	0.703
250	1.658 1.648	1.653	1.624
500	2.838 2.859	2.849	2.819

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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	108.93	6.05	5.6
2	20	204.09	11.31	5.5
3	20	442.97	13.98	3.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	108.82	3.65	3.4
2	24	226.91	12.24	5.4
3	24	458.76	20.82	4.5

Recovery

The recovery of IL-4 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	77	72-85
	1:4	80	70-114
Cell culture supernatants	1:2	101	88-123
	1:4	98	83-123

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Sample Values

Serum -Samples from volunteers were evaluated for human IL-4 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human serum (n=16)	18.9	31	ND-63.3 pg/mL
Human plasma serum (n=16)	10.6	25	ND-63 pg/mL

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were unstimulated or stimulated with 10 μg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of human IL-4.

Condition	Day1 (pg/mL)	Day5 (pg/mL)
Unstimulated	-	-
Stimulated	27	-

Sensitivity

The minimum detectable dose of human IL-4 is 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.

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Linearity

To assess the linearity of the assay, human plasma samples were spiked with high concentrations of IL-4 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. Cell culture supernatants samples were diluted with the appropriate **Sample Diluent PT1-ef** to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
No Dilutend	Average% of Expected		100
	Range (%)		—
1:2	Average% of Expected	98	95
	Range (%)	98-106	90-106
1:4	Average% of Expected	122	
	Range (%)	116-127	
1:8	Average% of Expected	111	
	Range (%)	109-114	
1:16	Average% of Expected	111	
	Range (%)	110-112	

References

1. Dhanda SK. et al.(2013) Clin Dev Immunol. doi: 10.1155/2013/263952.
2. Müller-Hermelink N. et al. (2008) Cancer Cell.13: 507-18.
3. Lebman DA. et al. (1988) 168: 853-62.
4. provided by RefSeq, Jul 2008.

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