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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL17A in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00015
Product Name	IL17A ELISA Kit
Species cross-reactivity	Human IL17A
Range (calibration Range)	62.5 - 4000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3605** (Human)
SwissProt	**Q16552** (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard -8000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-a- 30 mL/bottle; For serum, plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle; For cell culture supernatants	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

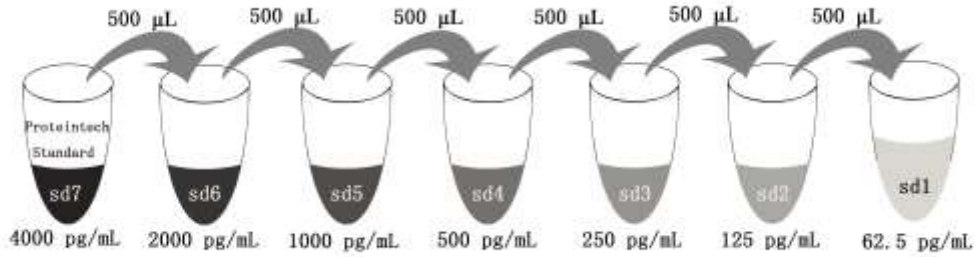
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-a is for standard, serum and plasma samples

Sample Diluent PT 1-ef is for standard and cell culture supernatants

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

*Add **2 mL** Sample Diluent PT 1-a or PT 1-ef in standard. This reconstitution gives a stock solution of 4000 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-a or 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

KE000015 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL17A ELISA kit is to be used to detect and quantify protein levels of endogenous IL17A . The assay recognizes human IL17A . An antibody specific for IL17A has been pre-coated onto the microwells. The IL17A protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for IL17A is added to detect the captured IL17A 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 450nm with the correction wavelength set at 630 nm.

background

IL17A, also named as IL-17, is a proinflammatory cytokine. IL-17, synthesized only by memory T cells and natural killer cells, has pleiotropic effects, mainly in the recruitment and activation of neutrophils. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis. The IL-17 receptor is a type I transmembrane protein, that is widely expressed on epithelial cells, fibroblasts, B and T cells, and monocytic cells. In psoriatic skin lesions, both Th17 cells and their downstream effector molecules, e.g. IL-17 and IL-22, are highly increased.

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

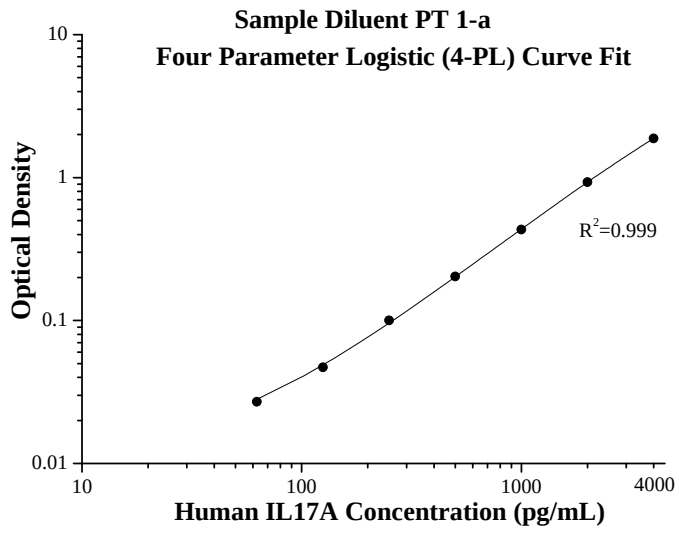
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

typical 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	0.042 0.044	0.043	—
62.5	0.071 0.069	0.07	0.027
125	0.091 0.089	0.09	0.047
250	0.142 0.143	0.1425	0.100
500	0.25 0.241	0.2455	0.203
1000	0.49 0.461	0.4755	0.433
2000	0.984 0.961	0.9725	0.930
4000	1.916 1.926	1.921	1.878

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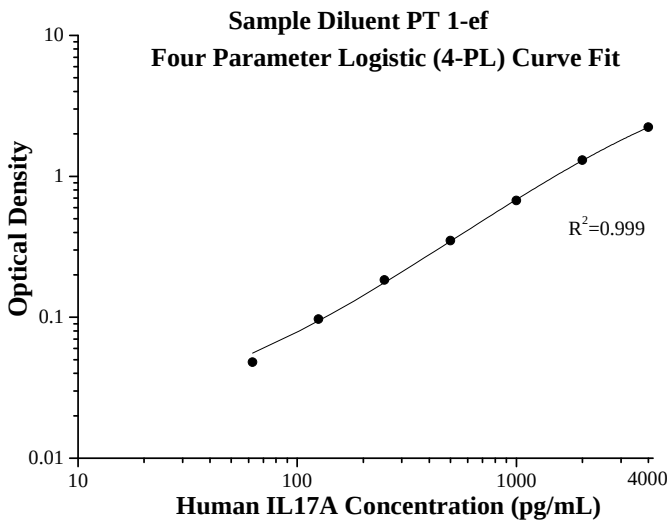

(pg/mL)	O.D	Average	Corrected
0	0.034	0.036	—
	0.038		
62.5	0.082	0.084	0.048
	0.086		
125	0.129	0.1325	0.097
	0.136		
250	0.216	0.2195	0.184
	0.223		
500	0.377	0.385	0.349
	0.393		
1000	0.713	0.7095	0.674
	0.706		
2000	1.336	1.336	1.300
	1.336		
4000	2.242	2.264	2.228
	2.286		

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	332.7	758.1	3086.8	333.0	813.8	3438.3
SD	17.0	24.2	91.4	21.0	49.5	268.4
CV%	5.1	3.2	3.0	6.3	6.1	7.8

recovery

The recovery of IL17A spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated (The samples were initially diluted 1:1).

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	100	85-120
	1:4	92	78-104
Cell culture supernatants	1:2	95	84-108
	1:4	88	83-96

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sample values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL17A in this assay. All samples measured less than the lowest standard, 62.5 pg/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human IL17A is 48 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 IL17A in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma (Sample Diluent PT 1-a)	Cell culture supernatants (Sample Diluent PT 1-ef)
1:2	Average% of Expected	94	98
	Range (%)	90-96	95-102
1:4	Average% of Expected	102	99
	Range (%)	99-104	94-103
1:8	Average% of Expected	102	98
	Range (%)	98-108	92-103
1:16	Average% of Expected	96	92
	Range (%)	92-103	90-95

references

1. T. Mabuchi. et al. (2012). J Dermatol Sci. 65: 4-11.
2. X. Song. et al. (2013). Cytokine. 62: 175-182.
3. E. Fitch. et al. (2007). Curr Rheumatol Rep. 9: 461-467.
4. provided by RefSeq, Jul 2008.

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