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AuthentiKine™

Human IL-17F Sandwich ELISA Kit Datasheet

For the quantitative detection of Human IL-17F in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00225
Product Name	AuthentiKine™ Human IL-17F Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.2-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	112744
SwissProt	Q96PD4

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 - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Additional Diluent AT 1-500 - Additional Diluent AT 1-500	1 bottle	
Sample Diluent PT 4 - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	1 bottle	
TSA concentrated solution (2000X) - 6 µL/vial	1vail	
TSA diluent buffer - 12 mL/bottle	1 bottle	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Detection Diluent - 30 mL/bottle	2 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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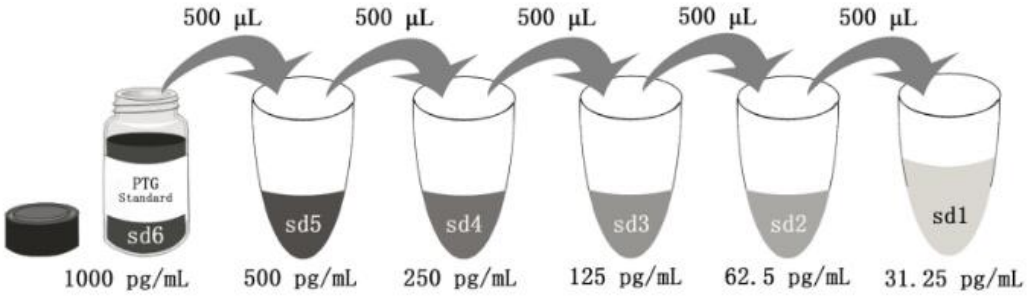
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4 is for protein standard, serum and plasma.
Sample Diluent PT 1-ef is for protein standard and cell culture supernatants samples.
Detection Diluent is for Detection antibody.
*Add **4 mL** Sample Diluent PT4 or PT 1-ef in protein standard. This reconstitution gives a stock solution of 2000 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 4 or PT 1-ef	**4000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00225 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-17F ELISA kit is to be used to detect and quantify protein levels of endogenous IL-17F. The assay recognizes human IL-17F. An antibody specific for IL-17F has been pre-coated onto the microwells. The IL-17F protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for IL-17F is added to detect the captured IL-17F 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

The interleukin 17 (IL-17) family of cytokines contains 6 structurally related cytokines, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. IL-17 family plays crucial roles in host defense against microbial organisms and in the development of inflammatory diseases. IL-17A is a pro-inflammatory cytokine that also has the capacity to promote angiogenesis and osteoclastogenesis. IL-17F shares the highest homology with IL-17A and signals via a receptor composed by the IL-17RA and IL-17RC subunits. IL-17A and IL-17F can form IL-17A/A or IL-17F/F homodimers, IL-17A/F heterodimers are also formed. IL-17A and IL-17F, produced by the Th17 CD4(+) T cell lineage, have been linked to a variety of inflammatory and autoimmune conditions. IL-17F levels are elevated in sera and lesional psoriatic skin compared to non-lesional tissue. IL-17F also has been implicated in the development of neutrophilic airway inflammation.

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Reagent Preparation

Amplification solution I

1. Prepare Amplification Solution I immediately prior to application on the plate.
2. Centrifuge vial for a few seconds in a microcentrifuge before opening to collect liquid trapped in the lid.
3. Dilute TSA censentrate (2000 X) in TSA diluent buffer to 1X working solution, for example: 3ul censentrate add to 6ml diluent buffer for 6trips, 6ul censentrate add to 12ml diluent buffer for 6trips
4. Discard immediately any prediluted Amplification Solution I after usage.

Amplification solution II

1. Prepare Amplification Solution II immediately prior to application on the plate.
2. Centrifuge vial for a few seconds in a microcentrifuge before opening to collect liquid trapped in the lid.
3. Dilute Streptavidin-horseradish peroxidase (HRP) (100X) in detection diluent to 1X working solution, for example: 60ul HRP add to 6ml detection diluent for 6trips, 120ul HRP add to 12ml detection diluent for 6trips
4. Discard immediately any prediluted Amplification Solution II after usage.

Sample Preparation

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

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 washDo not wash	Add additional diluent 50 µL per well then add standard and samples immediately
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	Amplification solution I	100 ul	15 min	4 times	Cover Wells incubate at room tempreture
5	Amplification solution II	100 ul	15 min	4 times	Cover Wells incubate at roo m tempreture
6	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
7	Stop Solution	100 µL	0 min	Do not wash	-
8	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

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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℃ 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℃ in a humid environment. Repeat the washes in step 6.

8. Signal development:

i. Add 100 µL of Amplification solution I (refer to "Reagent Preparation") to each well and incubated for 15 minutes at room tempreture, wash 4 times,Repeat the washes in step 6.

ii. Add 100 µL of Amplification solution II (refer to "Reagent Preparation") to each well and incubated for 15 minutes at room tempreture, wash 4 times ,Repeat the washes in step 6.

9. 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).

10. 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.

11. 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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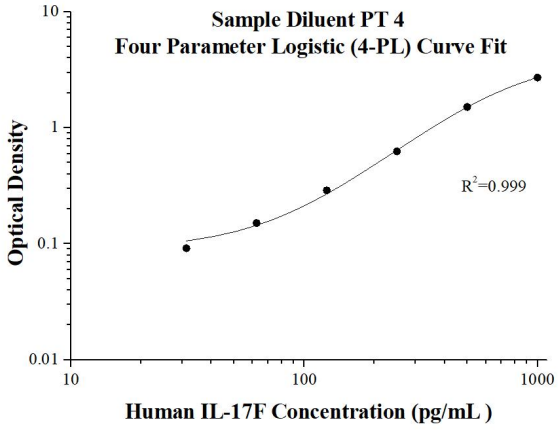
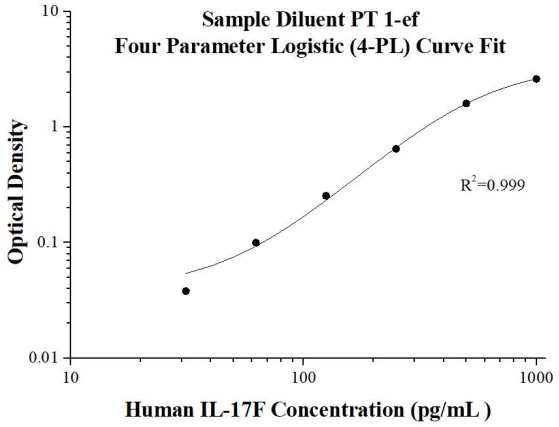
Example data

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	0.121 0.119	0.12	0
31.25	0.213 0.21	0.2115	0.0915
62.5	0.265 0.277	0.271	0.151
125	0.413 0.404	0.4085	0.2885
250	0.78 0.713	0.7465	0.6265
500	1.698 1.568	1.633	1.513
1000	2.84 2.892	2.8345	2.7145



(pg/mL)	O.D	Average	Corrected
0	0.15 0.135	0.1425	0
31.25	0.196 0.165	0.1805	0.038
62.5	0.245 0.239	0.242	0.0995
125	0.432 0.362	0.397	0.2545
250	0.795 0.785	0.79	0.6475
500	1.848 1.642	1.745	1.6025
1000	2.803 2.71	2.7565	2.614

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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	465.3	20.8	4.5
2	20	109.6	5.3	4.8
3	20	41.6	2.4	5.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	453.3	34.4	7.6
2	24	103.5	7.4	7.1
3	24	38.0	2.8	7.4

Recovery

The recovery of IL-17F 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	78	72-89
	1:4	82	74-89
Cell culture supernatants	1:2	93	79-106
	1:4	94	86-102

Sample Values

Serum - sixteen serum samples from healthy volunteers were evaluated for human IL-17F in this assay. fourteen samples measured less than the lowest standard, 15.6 pg/mL. Two samples measured 46.2 pg/mL and 277.7 pg/mL respectively. No medical histories were available for the donors used in this study.

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 1 μg/mL CD3 for 4days . Aliquots of the cell culture supernates were removed for levels of human IL-17F.

Condition	Day4 (pg/mL)
Unstimulated	-
Stimulated	3,282.8

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Sensitivity

The minimum detectable dose of human IL-17F is 14.4 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma (PT 4)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	81	100
	Range (%)	77-88	-
1:4	Average% of Expected	87	97
	Range (%)	81-94	89-104
1:8	Average% of Expected	90	105
	Range (%)	83-99	99-109
1:16	Average% of Expected	87	106
	Range (%)	83-93	96-117

References

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