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Antibodies | ELISA kits | Proteins

AuthentiKine®

Human IL-17F Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00225

Size: 96T

Sensitivity: 14.4 pg/mL

Range: 31.25-1000 pg/mL

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

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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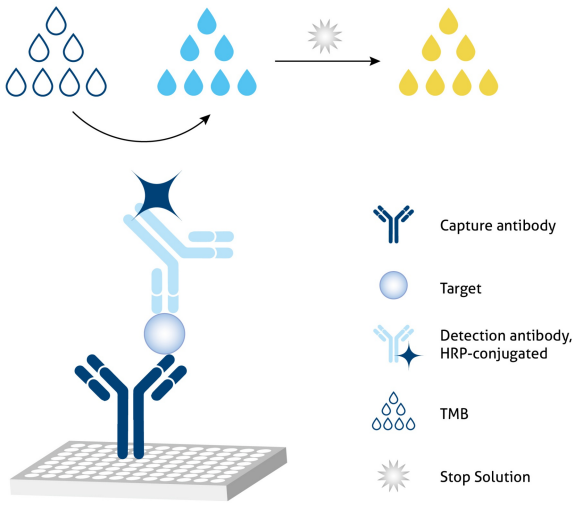
1. Background

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.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and 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 (100×) - 120 µL/vial*	1 vial	
Additional Diluent AT-00225 - 6 mL/bottle. Only for serum and plasma samples	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	
Amplification Reagent I (2000X) - 6 µL/vial*	1vail	
Amplification Diluent - 12 mL/bottle	1 bottle	
Amplification Reagent II (100×) - 120 µL/vial*	1 vial	
Detection Diluent - 30 mL/bottle	2 bottles	
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	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody, HRP-conjugated (1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution, HRP-conjugated for a few seconds prior to use)

7.3 Amplification solution I:

1. Prepare Amplification Solution I immediately prior to application on the plate.
2. Centrifuge vial for a fewsecondsin a microcentrifuge before opening to collect liquid trapped in the lid.
3. Dilute Amplification Reagent I (2000X) in Amplification Diluent to 1Xworking solution, for example: 3 µL concentrated solution add to 6 mL Amplification Diluent for 6 strips, 6 µL concentrated solution add to 12 mL Amplification Diluent for 12 strips.
4. Discard immediately any prediluted Amplification Solution I after usage.

7.4 Amplification solution II:

1. Prepare Amplification Solution II immediately prior to application on the plate.
2. Centrifuge vial for a fewsecondsin a microcentrifuge before opening to collect liquid trapped in the lid.
3. Dilute Amplification Reagent II (100×) in Detection Diluent to 1Xworking solution, for example: 60 µL Amplification Reagent II (100×) add to 6 mL Detection Diluent for 6 strips, 120 µL Amplification Reagent II (100×) add to 12 mL Detection Diluent for 12 strips.
4. Discard immediately any prediluted Amplification Solution II after usage.

7.5 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 is recommended for cell culture supernatant.

7.6 Standard Serial Dilution:

For human serum and plasma samples, add 4 mL Sample Diluent PT 4 in protein standard; For cell culture supernatant, add 2 mL Sample Diluent PT 1-ef in protein standard.

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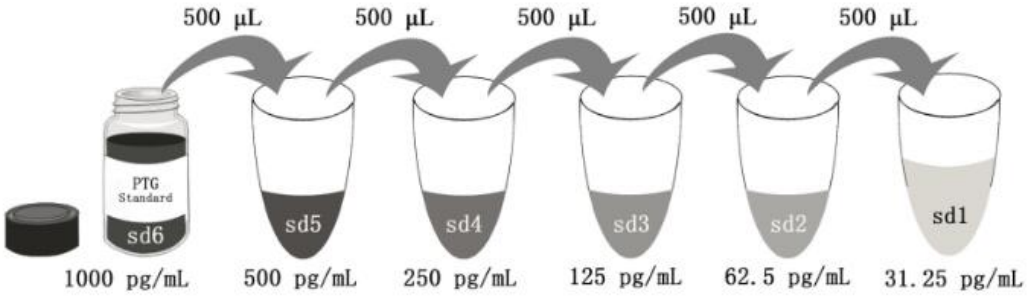

Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4 or PT 1-ef	**4000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group;

For serum or plasma , add 50 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant , no need to add Additional Diluent, directly follow the next step.

8.3. 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).

8.4 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) 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. Avoid getting any towel fibers in the wells or wells drying out completely.

8.6 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.7 Repeat wash step in 8.5.

8.8 Signal development:

1) Add 100 µL of Amplification solution I (refer to "Reagent Preparation 7.3") to each well and incubated for 15 minutes at room tempreture, wash 4 times,Repeat the washesin step 8.5.

2) Add 100 µL of Amplification solution II (refer to "Reagent Preparation 7.4") to each well and incubated for 15 minutes at room tempreture, wash 4 times,Repeat the washesin step 8.5

3) Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.9 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.10 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).

8.11 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

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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 Room 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.				

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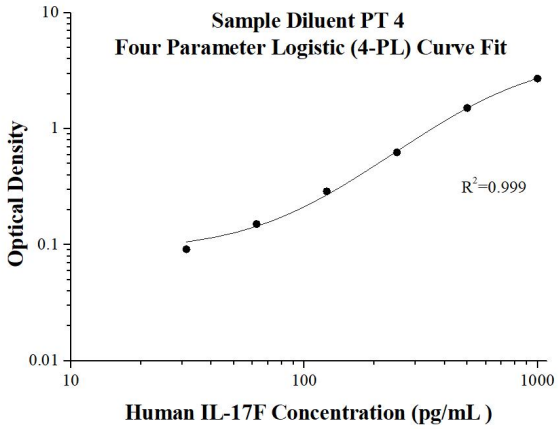
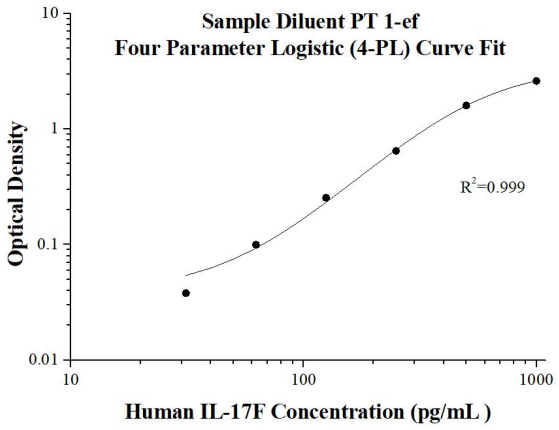
9. Validation Data

9.1 Standard curve

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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9.2 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

9.3 Recovery

The recovery of human IL-17F spiked to three different levels 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

9.4 Sample values

Serum - sixteen human 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 1µg/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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9.5 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.

9.6 Linearity

To assess the linearity of the assay, human serum and plasma samples were spiked with high concentrations of human IL-17F 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.

		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

10. References

1. Chunfang Gu. et al.(2013) Cytokine.64(2):477-85.
2. Seon Hee Chang. et al.(2009) Cytokine.46(1):7-11.
3. Askar M Akimzhanov. et al. (2021) J Biol Chem.282(9):5969-72.
4. Catherine Soderstrom. et al. (2017) AAPS J.19(4):1218-1222.
5. Jill F Wright. et al. (2008) J Immunol.181(4):2799-805.

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