

Human CCL17/TARC Sandwich ELISA Kit Datasheet

For the quantitative detection of Human CCL17/TARC in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00213
Product Name	Human CCL17/TARC Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8 - 500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	6361
SwissProt	Q92583

Kit Components & Storage

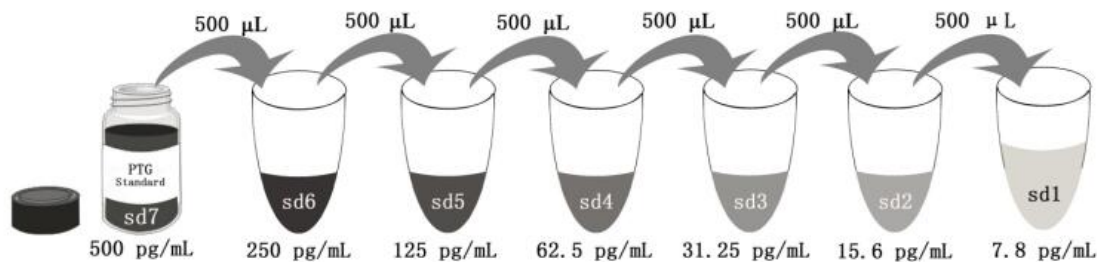
Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle.	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 is for protein standard and samples.

Detection Diluent is for Detection antibody and streptavidin-HRP.

*Add 2mL Sample Diluent PT 1 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	500 µL
# µL of Sample Diluent PT 1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00213 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CCL17/TARC ELISA kit is to be used to detect and quantify protein levels of endogenous CCL17/TARC. The assay recognizes human CCL17/TARC. An antibody specific for CCL17/TARC has been pre-coated onto the microwells. The CCL17/TARC protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human CCL17/TARC is added to detect the captured human CCL17/TARC protein. For signal development, Streptavidin-HRP 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 450 nm with the correction wavelength set at 630 nm.

Background

CCL17, also named as TARC, is a member of the CC chemokine family, and is highly expressed by thymus and other cells, including keratinocytes, endothelial cells, dendritic cells, bronchial epithelial cells and fibroblasts. CCL17 plays important roles in T cell development in thymus as well as in trafficking and activation of mature T cells. CCL17 induced chemotaxis and Ca²⁺ influx in the cells stably expressing CCR4, thereby demonstrating the ligand-receptor relationship between CCL17 and CCR4.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. 1:2 or 1:4 dilution is recommended for serum, plasma and cell culture supernatants samples.

Safety Notes

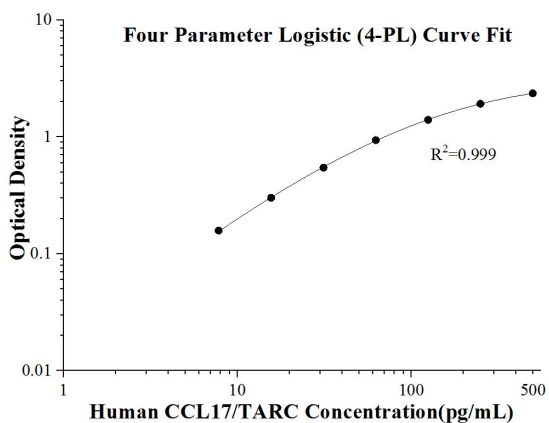
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

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 0.035	0.036	-
7.8	0.186 0.202	0.194	0.158
15.6	0.344 0.330	0.337	0.301
31.3	0.573 0.591	0.582	0.546
62.5	0.962 0.985	0.974	0.938
125	1.442 1.430	1.436	1.400
250	1.954 1.949	1.952	1.916
500	2.389 2.397	2.393	2.357

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	13.0	0.4	3.2
2	20	56.4	3.7	6.5
3	20	251.2	9.6	3.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	14.5	0.7	4.7
2	24	59.8	2.7	4.4
3	24	233.1	14.8	6.4

Recovery

The recovery of CCL17/TARC 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 serum	1:4	94	75-105
	1:8	89	78-99
Cell culture supernatants	1:4	100	84-116
	1:8	101	75-115

Sample Values

Serum/Plasma

Serum and plasma samples from healthy volunteers were evaluated for CCL17/TARC in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=32)	169.2	21.6-513.1

Cell culture supernatants

Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernatants were removed on days 1 and 5 and assayed for levels of human TARC.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	6.3	50.7
Stimulated	143.3	994.6

Sensitivity

The minimum detectable dose of human CCL17/TARC is 2.8 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, human serum, cell lysates were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	102	90
	Range (%)	99-106	87-108
1:8	Average% of Expected	103	80
	Range (%)	86-111	77-106
1:16	Average% of Expected	101	82
	Range (%)	94-110	89-110

References

1. T Imai. et al. (1997) J Biol Chem. 272(23):15036-42.
2. Hidehisa Saeki. et al. (2006) J Dermatol Sci. 43(2):75-84.
3. Kashif Rasheed. et. al.(2018) Oncotarget. 9(59):31432-31447.