Human CCL4 Sandwich ELISA Kit Datasheet

For the quantitative detection of human CCL4 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00152
Product Name	Human CCL4 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	6351
SwissProt	P13236

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, biotinylated(100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 5 - 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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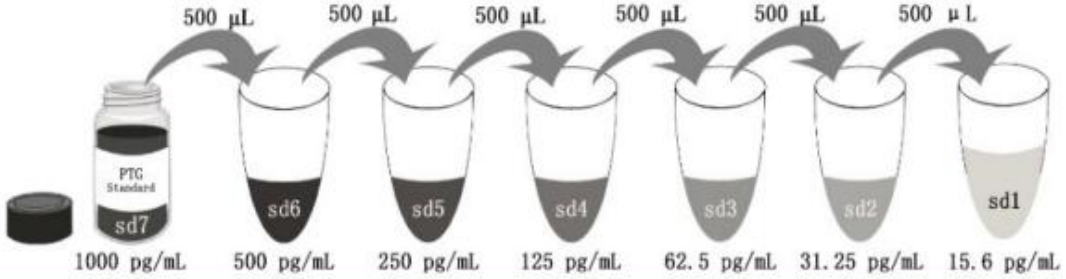
NB: Do not use the kit after the expiration date.
Sample Diluent PT 5 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 5 in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



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 5	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00152 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CCL4 ELISA kit is to be used to detect and quantify protein levels of endogenous CCL4. The assay recognizes human CCL4. An antibody specific for CCL4 has been pre-coated onto the microwells. The CCL4 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human CCL4 is added to detect the captured human CCL4 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

CCL4(C-C chemokine ligand 4), also known as MIP-1β, plays a key role in inflammation and immune regulation. CCL4 is produced by a wide variety of cells including: immune cells (monocytes, B and T cells), fibroblasts, endothelial, and epithelial cells. CCL4 has a broad spectrum of target cells including immature dendritic cells, which express the cognate receptor CCR5. Increased expression of CCL4 was reported in the monocytes and blood of elderly individuals. Serum concentrations of CCL4 have been found to be significantly higher in patients with head and neck squamous cell carcinoma compared with controls.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:2 or 1:4 before assay, and 1:80 or 1:160 dilution is recommended for cell culture supernatants.

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Safety Notes

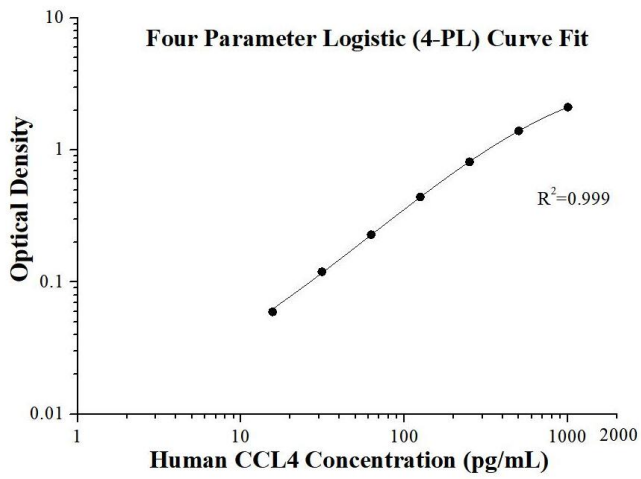
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

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	0.103 0.107	0.105	-
15.62	0.158 0.171	0.164	0.059
31.25	0.212 0.237	0.224	0.119
62.5	0.314 0.353	0.333	0.228
125	0.522 0.571	0.546	0.441
250	0.889 0.948	0.918	0.813
500	1.489 1.524	1.505	1.400
1000	2.165 2.273	2.219	2.114

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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	436.0	38.1	8.7
2	20	102.2	9.8	9.6
3	20	60.6	5.5	9.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	417.7	28.1	6.7
2	24	115.5	5.7	5.0
3	24	62.2	6.7	10.7

Recovery

The recovery of CCL4 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	1:2	104	86-121
	1:4	84	75-95
Cell culture supernatants	1:320	100	87-112
	1:640	85	74-100

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Sample Values

Serum samples from healthy volunteers (human) were evaluated for CCL4 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=16)	184.9	16.6-366.0

Human peripheral blood leuocytes cells (1 x 10^6cells/mL) were cultured in DMEM supplemented with 8% fetal bonive serum, 5 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 1 day. Aliquots of the cell culture supernatants were removed and assayed for levels of human CCL4.

Condition	Day 1 (pg/mL)
Unstimulated	7,537
Stimulated	26,367

Sensitivity

The minimum detectable dose of human CCL4 is 3.4 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 serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	97	105
	Range (%)	92-107	100-108
1:8	Average% of Expected	87	111
	Range (%)	80-94	102-116
1:16	Average% of Expected	-	110
	Range (%)	-	97-121

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References

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