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colorimetric sandwich ELISA kit datasheet

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

general information

Catalogue Number	KE00062
Product Name	CCL4 ELISA Kit
Species cross-reactivity	Human CCL4
Range (calibration Range)	7.8 - 500 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**6351** (Human)
SwissProt	**P13236** (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 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	

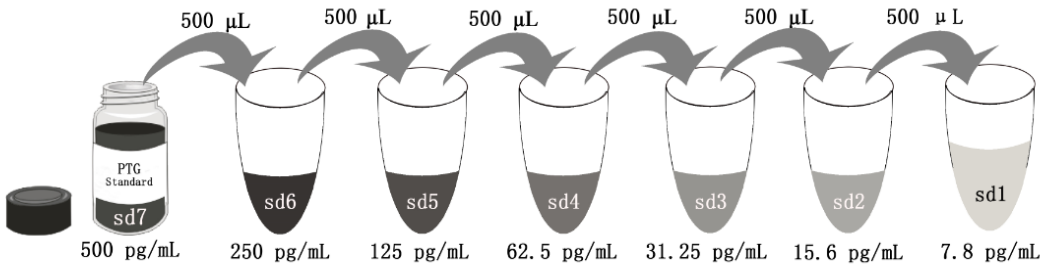
NB: Do not use the kit after the expiration date.

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

Sample Diluent PT 1-ef is for standard, 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 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-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

KE00062 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. A polyclonal 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, a monoclonal antibody specific for CCL4 is added to detect the captured CCL4 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.

background

CCL4, also known as Macrophage inflammatory protein-1β (MIP-1β), is a CC chemokine with specificity for CCR5 receptors, and is one of the major HIV-suppressive factors produced by CD8+ T-cells. MIP-1β is an acidic protein composed of 69 amino acids that is produced by many cells, particularly macrophages, dendritic cells, and lymphocytes. MIP-1β is responsible for the activation of PMN and is involved in acute neutrophilic inflammation. Recombinant MIP-1β induces a dose-dependent inhibition of different strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV).

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

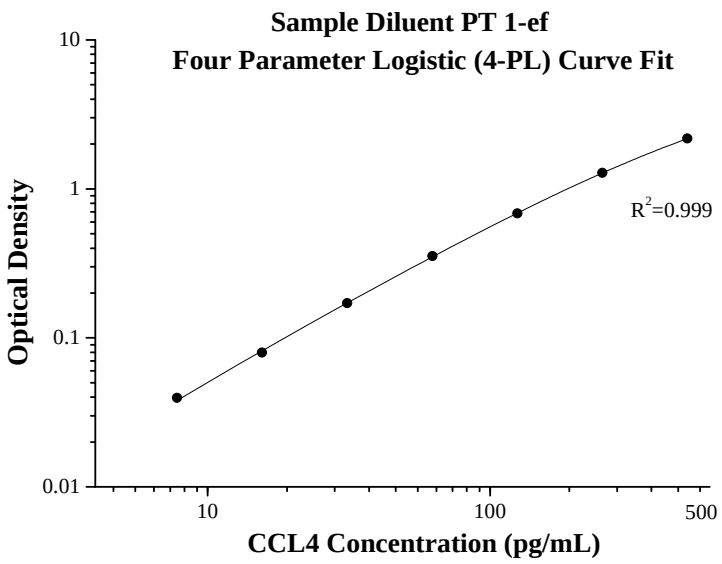
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
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	0.02	0.021	—
	0.022		
7.8	0.063	0.0605	0.0395
	0.058		
15.6	0.101	0.1005	0.0795
	0.1		
31.25	0.199	0.192	0.171
	0.185		
62.5	0.381	0.375	0.354
	0.369		
125	0.706	0.7055	0.6845
	0.705		
250	1.29	1.301	1.28
	1.312		
500	2.188	2.196	2.175
	2.204		

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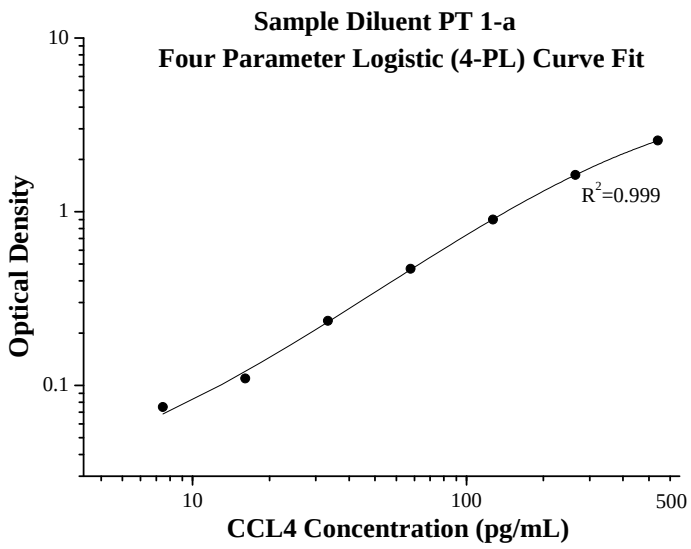

(pg/mL)	O.D	Average	Corrected
0	0.017	0.0175	—
	0.018		
7.8	0.093	0.092	0.075
	0.092		
15.6	0.129	0.127	0.1095
	0.125		
31.25	0.261	0.2525	0.235
	0.244		
62.5	0.482	0.4865	0.469
	0.491		
125	0.935	0.918	0.9005
	0.901		
250	1.654	1.647	1.6295
	1.64		
500	2.58	2.582	2.5645
	2.584		

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)	388.3	102.8	25.7	391.2	101.8	26.2
SD	10	3.8	0.7	13.8	2.7	1.3
CV%	2.6	3.7	2.8	3.5	2.6	4.8

recovery

The recovery of CCL4 spiked to three different levels in four samples throughout the range of the assay in vrious matrices was evaluated.

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:2	108	100-114
	1:4	113	107-119
Cell culture supernatants	1:2	98	96-101
	1:4	104	92-113

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sensitivity

The minimum detectable dose of human CCL4 is 1 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 CCL4 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	81	109
	Range(%)	80-82	103-113
1:4	Average% of Expected	91	104
	Range(%)	87-95	102-106
1:8	Average% of Expected	91	102
	Range(%)	88-95	98-107
1:16	Average% of Expected	92	101
	Range(%)	86-100	95-108

references

1. Bystry RS. et al. (2001). Nat Immunol. 2:1126-32.
2. Cocchi F. et al.(1995). Science. 270:1811-5.
3. Kamin-Lewis R. et al. (2001). Proc Natl Acad Sci U S A. 98:9283-8.
4. provided by RefSeq, Dec 2012.