

## Mouse CCL12/MCP-5 Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse CCL12/MCP-5 in serum, plasma and cell culture supernatants samples.

### General Information

Catalogue Number	KE10051
Product Name	Mouse CCL12/MCP-5 Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	3.9-250 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	20293 (mouse)
SwissProt	Q62401 (mouse)

### Kit Components & Storage

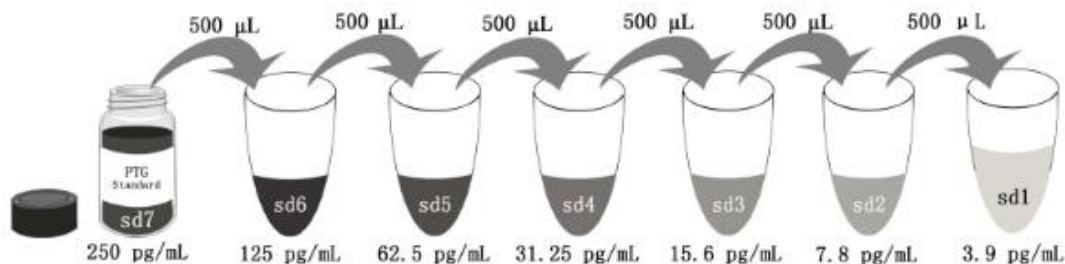
Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 250 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3 - 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	

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

Sample Diluent PT 3 is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1 mL Sample Diluent PT 3 in protein standard. This reconstitution gives a stock solution of 250 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 3	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE10051 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CCL12/MCP-5 ELISA kit is to be used to detect and quantify protein levels of endogenous CCL12/MCP-5. The assay recognizes mouse CCL12/MCP-5. An antibody specific for CCL12/MCP-5 has been pre-coated onto the microwells. The CCL12/MCP-5 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse CCL12/MCP-5 is added to detect the captured mouse CCL12/MCP-5 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

CCL12, also known as MCP-5, is a small cytokine belonging to the CC chemokine family. It is a structural and functional homologue of human MCP-1. CCL12 is found predominantly in lymph nodes and thymus under normal conditions, and its expression can be markedly induced in activated macrophages both in vitro and in vivo. CCL12 is involved in allergic inflammation and the host response to pathogens and may play a pivotal role during early stages of allergic lung inflammation.

## 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 or plasma, 1:2 or 1:4 dilution is recommended for cell culture supernatants.

## Safety Notes

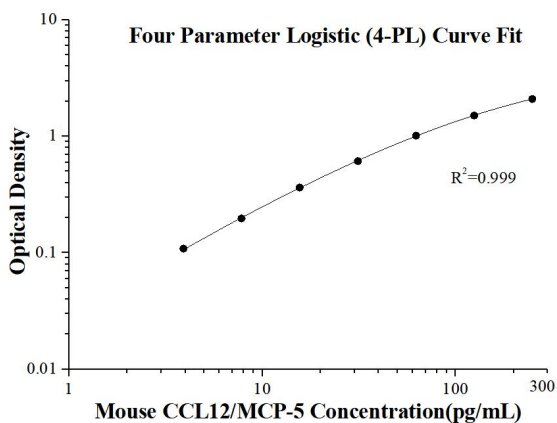
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	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	0.034 0.028	0.031	0
3.9	0.139 0.140	0.14	0.109
7.8	0.226 0.230	0.228	0.197
15.6	0.396 0.392	0.394	0.363
31.25	0.635 0.653	0.644	0.613
62.5	1.055 1.037	1.046	1.015
125	1.553 1.529	1.541	1.51
250	2.154 2.097	2.126	2.095

## 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	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	116.3	2.9	2.5	1	24	115.0	2.3	2.0
2	20	26.8	0.9	3.4	2	24	26.1	0.6	2.4
3	20	5.5	0.1	2.0	3	24	6.2	0.2	3.3

## Recovery

The recovery of CCL12/MCP-5 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 (%)
Mouse serum	1:8	90	85-97
	1:16	89	80-94
Cell culture supernatants	1:8	98	91-107
	1:16	95	85-112

## Sample Values

Mouse serum samples were evaluated for the presence of mouse CCL12/MCP-5 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse serum (n=15)	40.9	30.0-56.0

### Cell Culture Supernates:

Mouse splenocytes ( $1 \times 10^6$  cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum supplemented with 50  $\mu$  M  $\beta$ -mercaptoethanol and 10 ng/mL recombinant human IL-2. An aliquot of the cell culture supernate was removed, assayed for mouse MCP-5, and measured 134 pg/mL.

## Sensitivity

The minimum detectable dose of mouse CCL12/MCP-5 is 0.06 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, mouse serum, cell culture supernatants were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	104	109
	Range (%)	101-108	107-112
1:8	Average% of Expected	108	113
	Range (%)	106-110	110-117
1:16	Average% of Expected	111	114
	Range (%)	102-118	111-117

## References

1. M N Sarafi. et al. (1997) J Exp Med.185(1):99-109.
2. D.A. Luster. et al. (1998) J Exp Med. 338 (1998), 436-445.
3. G Q Jia. et al. (1996) J Exp Med. 184(5):1939-51.