

Mouse CX3CL1 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE10076

Size: 96T

Sensitivity: 0.09 ng/mL

Range: 0.625-20 ng/mL

Usage: For the quantitative detection of mouse CX3CL1 concentrations in serum, plasma, cell culture supernatant and tissue lysate.

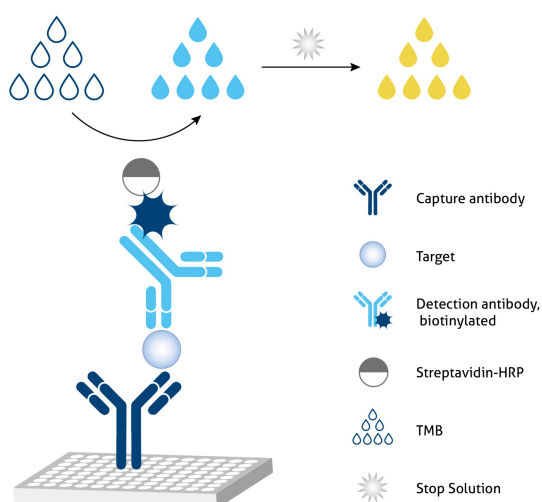
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1. Background

Chemokines direct the trafficking of white blood cells in immune surveillance, playing a key role in inflammatory and infectious diseases such as AIDS. CX3CL1, also known as fractalkine (FKN), is a unique, membrane-bound chemokine, with a transmembrane domain and the chemokine domain on top of a long mucinlike stalk. This protein can exist in two forms, a membrane-bound form or a shed soluble form, after extracellular proteolysis at a membrane-proximal dibasic cleavage site. The soluble CX3CL1 has potent chemoattractant activity for T cells and monocytes, and the cell-surface-bound protein, which is induced by primary proinflammatory signals in activated primary endothelial cells, promotes strong adhesion of those leukocytes. Presence of CX3CL1 and its receptor CX3CR1 in lymphocytes and their enhanced cellular expression during inflammatory conditions suggests that the CX3CL1-CX3CR1 duo participates in immune-related inflammatory diseases.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. 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 Distilled or deionized water.
- 3.4 Plate washer: automated or manual.
- 3.5 Absorbent paper towels.
- 3.6 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.7 Beakers and graduated cylinders.
- 3.8 100 mM PMSF (phenylmethanesulfonyl fluoride) stock solution (1g PMSF in 57 mL isopropyl alcohol), only for lysate sample.
- 3.9 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.

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 - 40 ng/bottle; lyophilized	2 bottles	
Detection Antibody (100×), biotinylated - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 3-ec - 30 mL/bottle. For mouse serum and plasma	1 bottle	
Sample Diluent PT 3-ag - 30 mL/bottle. For cell culture supernatant and tissue lysate	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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

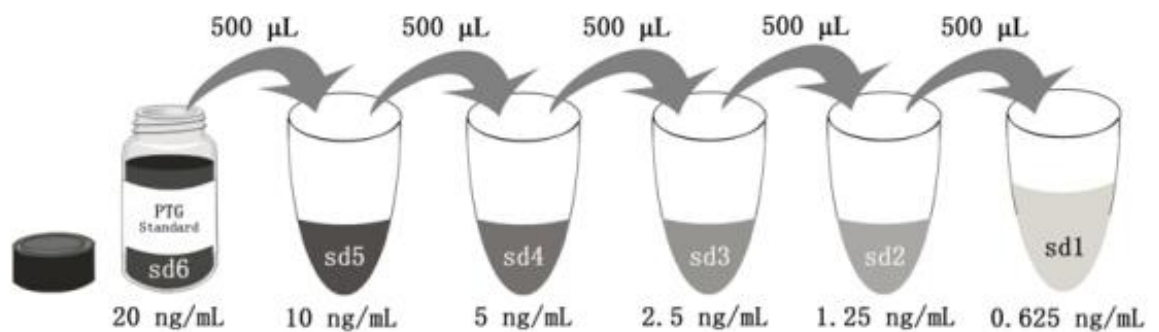
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 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 mouse serum and plasma; 1:2 is recommended for cell culture supernatant; 1:16 or 1:32 is recommended for tissue lysate.

7.5 Standard Serial Dilution:

For mouse serum and plasma samples, add 2 mL Sample Diluent PT 3-ec in protein standard. For cell culture supernatants and tissue lysates, add 2 mL Sample Diluent PT 3-ag in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-ec or PT 3-ag	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP 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, 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, it is recommended to assay all standards, controls, and samples in duplicate).

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

8.4 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.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: 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.

8.10 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.

8.11 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).

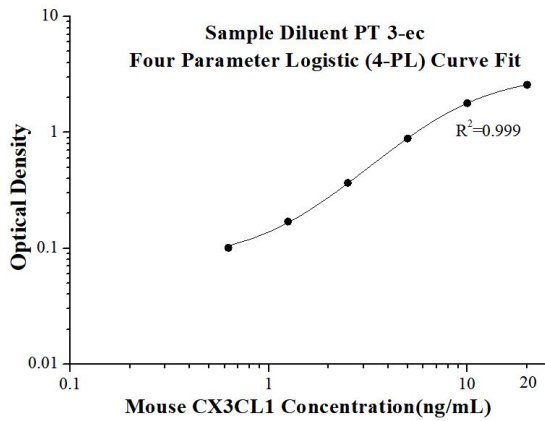
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use 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.

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.				

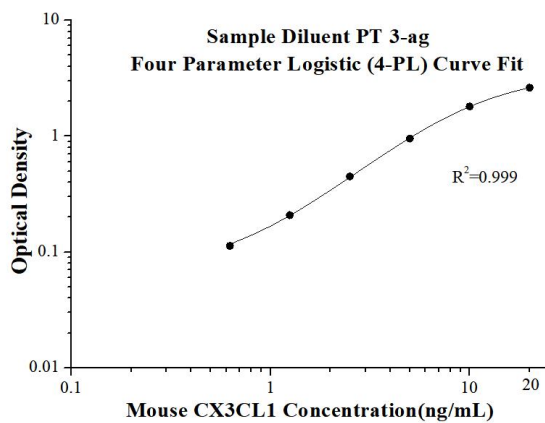
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.101 0.105	0.103	-
0.625	0.138 0.143	0.141	0.101
1.25	0.205 0.214	0.210	0.170
2.5	0.401 0.413	0.407	0.367
5	0.891 0.962	0.927	0.887
10	1.840 1.821	1.831	1.791
20	2.618 2.605	2.612	2.572



(ng/mL)	O.D	Average	Corrected
0	0.099 0.097	0.098	-
0.625	0.154 0.152	0.153	0.113
1.25	0.248 0.248	0.248	0.208
2.5	0.490 0.488	0.489	0.449
5	0.993 0.995	0.994	0.954
10	1.849 1.832	1.841	1.801
20	2.650 2.661	2.656	2.616

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					Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%	Sample	n	Mean (ng/mL)	SD	CV%
1	20	9.63	0.41	4.29	1	24	9.64	0.37	3.89
2	20	2.34	0.13	5.70	2	24	2.40	0.09	3.66
3	20	1.27	0.03	2.54	3	24	1.23	0.08	6.47

9.3 Recovery

The recovery of mouse CX3CL1 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:2	96	79-111
	1:4	96	78-110
Cell culture supernatant	1:4	96	88-111
	1:8	94	79-106
Tissue lysate	1:20	88	82-97
	1:40	86	77-93

9.4 Sample values

Mouse serum - The mouse serum samples were evaluated for the presence of mouse CX3CL1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Mouse serum (n=24)	1.82	0.42-3.38

Cell culture supernatant - L-929 mouse fibroblast cells were cultured for 3 days in MEM supplemented with 10% equine serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. An aliquot of the cell culture supernatant was removed, assayed for mouse CX3CL1, and measured 9.35 ng/mL.

Tissue lysate - Mouse brain tissue lysates were evaluated for the presence of mouse CX3CL1 in this assay, and measured 78.83 ng/mL.

9.5 Sensitivity

The minimum detectable dose of mouse CX3CL1 is 0.09 ng/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, mouse serum were spiked with high concentrations of mouse CX3CL1 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant and tissue lysate was diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The tissue lysate samples were initially diluted 1:8)

		Mouse serum (Sample Diluent PT 3-ec)	Cell culture supernatant (Sample Diluent PT 3-ag)	Tissue lysate (Sample Diluent PT 3-ag)
1:2	Average% of Expected	96	100	100
	Range (%)	94-98	-	-
1:4	Average% of Expected	101	104	112
	Range (%)	100-102	100-109	109-114
1:8	Average% of Expected	111	108	124
	Range (%)	102-115	101-117	123-124
1:16	Average% of Expected	103	100	118
	Range (%)	89-113	92-109	113-123

10. References

1. Bazan JF, et al. A new class of membrane-bound chemokine with a CX3C motif. *Nature*. 385(6617):640-4 (1997).
2. Fraticelli P, et al. Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. *J Clin Invest*. 107(9):1173-81 (2001).
3. Jones BA, et al. Fractalkine/CX3CL1: a potential new target for inflammatory diseases. *Mol Interv*. 10(5):263-70 (2010).