Human CCL7/MCP-3 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00262

Size: 5*96T

Sensitivity: 1.4 pg/mL

Range: 7.8-500 pg/mL

Usage: For the quantitative detection of human CCL7/MCP-3 concentrations in serum, plasma and cell culture supernatant.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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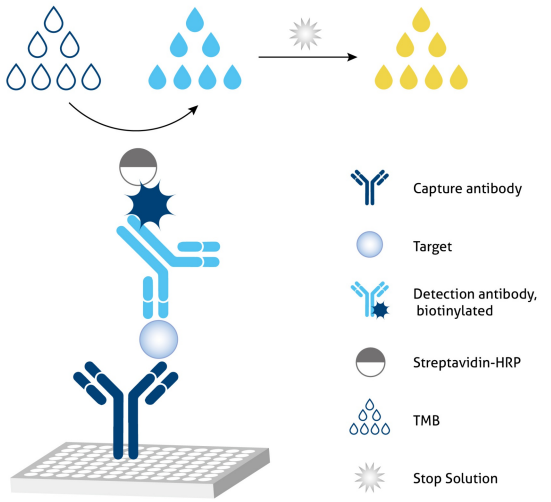
1. Background

The chemokine CCL7 (MCP3) is a chemotactic factor and potent attractant of monocytes firstly characterized from the culture supernatants of MG-63 osteosarcoma cells. CCL7 is known to promote the recruitment of many innate immune cell types including monocytes and neutrophils to sites of bacterial and viral infection and eosinophils and basophils to sites of allergic inflammation. CCL7 is expressed at low levels in endothelial cells, fibroblasts and mononuclear cells and upregulated by various stimuli including viruses, type I or type II interferons (IFNs). CCL7 (MCP-3) mediates effects on a host of innate and adaptive immune cell types through binding to numerous receptors including CCR1, CCR2, CCR3, CCR5, and CCR10. Various studies have shown that tumor cells and stromal cells also produce high levels of CCL7, while the specific response element and signaling pathways involved are not entirely clear.

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 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 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.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	**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 - 2000 pg/bottle; lyophilized	10 bottles	
Detection Antibody (100×), biotinylated - 600 μL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 600 μL/vial*	1 vial	
Sample Diluent PT 4 - 150 mL/bottle	1 bottle	
Detection Diluent - 150 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
Stop Solution - 60 mL/bottle	1 bottle	
Plate Cover Seals	15 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℃. 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℃. 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 ≤ -20℃. Avoid repeated freeze-thaw cycles.

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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).

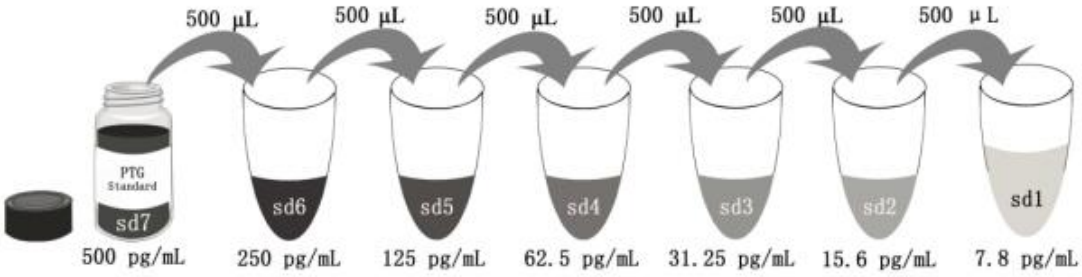
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Streptavidin-HRP + 990 μL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

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 or 1:4 is recommended for human serum and plasma; 1:5 or 1:10 is recommended for cell culture supernatant.

7.5 Standard Serial Dilution:

Add 4 mL Sample Diluent PT 4 in protein standard.



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 4	**4000 μL**	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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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 Preparation7.2) 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 Preparation7.3) 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.				

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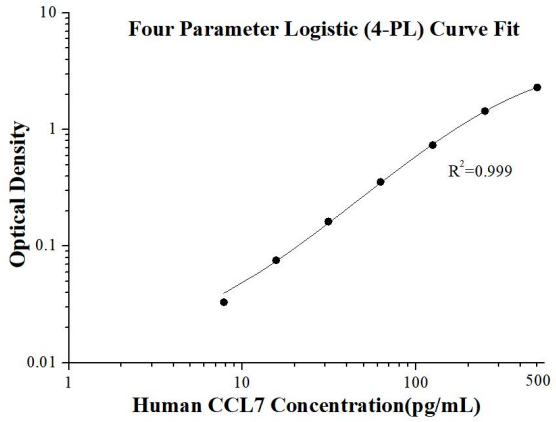
9. Validation Data

9.1 Standard curve

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



(pg/mL)	O.D	Average	Corrected
0	0.068 0.066	0.067	-
7.8	0.099 0.101	0.1	0.033
15.6	0.14 0.145	0.1425	0.0755
31.25	0.232 0.227	0.2295	0.1625
62.5	0.418 0.427	0.4225	0.3555
125	0.803 0.802	0.8025	0.7355
250	1.495 1.521	1.508	1.441
500	2.389 2.343	2.366	2.299

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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	13.3	0.5	3.4
2	20	57.1	1.0	1.8
3	20	218.7	4.9	2.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	13.8	1.0	7.3
2	24	55.3	1.0	1.8
3	24	210.0	7.6	3.6

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9.3 Recovery

The recovery of human CCL7 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	78	74-81
	1:4	97	91-100
Cell culture supernatant	1:10	106	94-114
	1:20	103	95-110

9.4 Sample values

Human serum -human serum samples were evaluated for the presence of human CCL7 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=20)	24.8	14.4-46.2

Cell culture supernatant-Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. Cells were stimulated with 10 ug/mL PHA. Aliquots of the cell culture supernatant was removed on days 1 and day 5 assayed for levels of human CCL7.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND*	ND*
Stimulated	2,177.9	16,138.8

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human CCL7 is 1.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, human serum samples were spiked with high concentrations of human CCL7 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatant was diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatant
1:2	Average% of Expected	90	100
	Range (%)	79-97	-
1:4	Average% of Expected	106	100
	Range (%)	95-112	93-106
1:8	Average% of Expected	113	98
	Range (%)	110-117	90-102
1:16	Average% of Expected	120	96
	Range (%)	116-124	87-102

10. References

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