

Human CCL22 ELISA Kit User Manual

Catalog # CEK1042

(Version 1.1A)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative
Detection of Human CCL22 Concentrations in Cell Culture
Supernatants, Serum, Plasma.

For research use only. Not for diagnostic or therapeutic procedures.



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I. INTRODUCTION

Macrophage-derived chemokine (MDC), also called Chemokine, cc motif, ligand 22 (CCL22) or Small inducible cytokine subfamily A, member 22 (SCY22). MDC is a recently identified member of the CC chemokine family. It is not closely related to other chemokines, sharing most similarity with thymus- and activation-regulated chemokine (TARC), which contains 37% identical amino acids. In addition, MDC gene is mapped to chromosome 16q13, the same position reported for the TARC gene. MDC has the four-cysteine motif and other highly conserved residues characteristic of CC chemokines, but it shares <35% identity with any of the known chemokines. Recombinant MDC was expressed in Chinese hamster ovary cells and purified by heparin-Sepharose chromatography. MDC is highly expressed in macrophages and in monocyte-derived dendritic cells, but not in monocytes, natural killer cells, or several cell lines of epithelial, endothelial, or fibroblast origin. High expression was also detected in normal thymus and less expression in lung and spleen. MDC is thus a unique member of the CC chemokine family that may play a fundamental role in the function of dendritic cells, natural killer cells, and monocytes.



II. ASSAY PRINCIPLES

The Cohesion Bioscience Human CCL22 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human CCL22 in Cell Culture Supernatants, Serum, Plasma. This assay employs an antibody specific for Human CCL22 coated on a 96-well plate. Standards and samples are pipetted into the wells and CCL22 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human CCL22 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CCL22 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.



III. KIT COMPONENTS

Component	Volume
96-well Plate Coated with Anti-Human CCL22 Antibody	8 wells x 12 Strips
Human CCL22 Standard	1 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 μΙ
Streptavidin-HRP (100X)	120 μΙ
Standard/Sample Diluent	30 ml
Detection Antibody Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

IV. STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.



V. MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Microplate reader capable of measuring absorbance at 450 nm.
- 2. Adjustable pipettes and pipette tips to deliver 2 μ l to 1 ml volumes.
- 3. Adjustable 1-25 ml pipettes for reagent preparation.
- 4. 100 ml and 1 liter graduated cylinders.
- 5. Absorbent paper.
- 6. Distilled or deionized water.
- 7. Computer and software for ELISA data analysis.
- 8. Tubes to prepare standard or sample dilutions.

VI. HEALTH AND SAFETY PRECAUTIONS

- 1. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- 2. Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.



VII. REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 X g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

Cell Lysates: Collect cells and rinse cells with PBS. Homogenize and lyse cells throughly in lysate solution. Centrifuge cell lysates at approximately 10000 X g for 5 minutes to remove debris. Aliquots of the cell lysates were removed and assayed.

Bone Tissue: Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M Guanidine-HCl.

Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at \leq -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed. Alternatively, aliquot and store samples at \leq -20 °C.

Note: Some lysis buffer, such as RIPA can not be used. Some components will affect the binding.

Urine: Urinary samples should be cleared by centrifugation and then can be used directly without dilution. Storage at -20°C.



2. Human CCL22 Standard Preparation

Reconstitute the lyophilized Human CCL22 Standard by adding 1 ml of Standard/Sample Diluent to make the 1000 pg/ml standard stock solution. Allow solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Two tubes of the standard (1 ng per tube) are included in each kit. Use one tube for each experiment.

Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (15.6 pg/ml - 1000 pg/ml) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 pg/ml).

Standard	Add	Into
1000 pg/ml		
500 pg/ml	500 μl of the Standard (1000 pg/ml)	500 μl of the Standard/Sample Diluent
250 pg/ml	500 μl of the Standard (500 pg/ml)	500 μl of the Standard/Sample Diluent
125 pg/ml	500 μl of the Standard (250 pg/ml)	500 μl of the Standard/Sample Diluent
62.5 pg/ml	500 μl of the Standard (125 pg/ml)	500 μl of the Standard/Sample Diluent
31.2 pg/ml	500 μl of the Standard (62.5 pg/ml)	500 μl of the Standard/Sample Diluent
15.6 pg/ml	500 μl of the Standard (31.2 pg/ml)	500 μl of the Standard/Sample Diluent
0 pg/ml	1 ml of the Standard/Sample Diluent	

Note: The standard solutions are best used within 2 hours. The 1000 pg/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- 3. Biotin-Labeled Detection Antibody Working Solution Preparation

 The Biotin-Labeled Detection Antibody should be diluted in 1:100 with the Detection

 Antibody Diluent and mixed thoroughly. The solution should be prepared no more than 2 hours prior to the experiment.
- 4. Streptavidin-HRP Working Solution Preparation



The Streptavidin-HRP should be diluted in 1:100 with the Streptavidin-HRP Diluent and mixed thoroughly. The solution should be prepared no more than 1 hour prior to the experiment.

5. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 600 ml with glass-distilled or deionized water (1:20).



VIII. ASSAY PROCEDURE

The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of protein amount in samples.

- 1. Add 100 µl of each standard and sample into appropriate wells.
- 2. Cover well and incubate for 90 minutes at room temperature or over night at 4°C with gentle shaking.
- 3. Remove the cover, discard the solution and wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
- 4. Add 100 μ l of Biotin-Labeled Detection Antibody Working Solution into each well and incubate the plate at 37°C for 60 minutes.
- 5. Wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 2 minutes. Discard the Wash Buffer Working Solution and blot the plate onto paper towels or other absorbent material.
- 6. Add 100 μ l of Streptavidin-HRP Working Solution into each well and incubate the plate at 37°C for 45 minutes.
- 7. Wash plate 5 times with Wash Buffer Working Solution, and each time let wash buffer stay in the wells for 1 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.
- 8. Add 100 μ l of TMB Substrate Solution into each well and incubate plate at 37°C in dark for 10-20 minutes.
- 9. Add 100 μ l of Stop Solution into each well. The color changes into yellow immediately.



10. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.



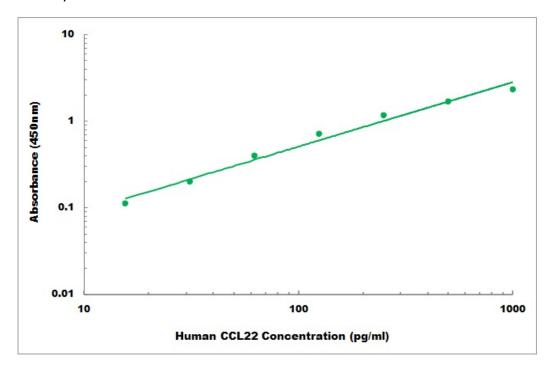
IX. ASSAY PROCEDURE SUMMARY

M	Prepare all reagents, samples and standards
M	• Add 100 μl Standard or Sample
M	Wash plate 3 times with Wash Buffer Working Solution
M	• Add 100 μl Biotin-Labeled Detection Antibody Working Solution
M	Wash plate 3 times with Wash Buffer Working Solution
M	• Add 100 μl Streptavidin-HRP Working Solution
M	Wash plate 5 times with Wash Buffer Working Solution
M	• Add 100 μl TMB Substrate Solution
M	• Add 100 μl Stop Solution
	• Read the plate at 450nm



X. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



XI. SENSITIVITY

The minimum detectable dose of Human CCL22 is typically less than 8 pg/ml.

XII. SPECIFICITY

The Human CCL22 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human CCL22 proteins within the range of 15.6 pg/ml - 1000 pg/ml.



XIII. CROSS REACTIVITY

No detectable cross-reactivity with other relevant proteins.

XIV. REFERENCES

1. Imai, T.; Chantry, D.; Raport, C. J.; Woods, C. L.; Nishimura, M.; Godiska, R.; Yoshie, O.; Gray, P. W.: Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J. Biol. Chem. 273: 1764-1768, 1998.

2. Godiska, R.; Chantry, D.; Raport, C. J.; Sozzani, S.; Allavena, P.; Leviten, D.; Mantovani, A.; Gray, P. W.: Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells. J. Exp. Med. 185: 1595-1604, 1997.



XV. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
High signal and background in	Insufficient washing	Increase number of washes
all wells		Increase time of soaking
		between in wash
	Too much Streptavidin-HRP	Check dilution, titration
	Incubation time too long	Reduce incubation time
	Development time too long	Decrease the incubation
		time before the stop solution
		is added
No signal	Reagent added in incorrect	Review protocol
	order, or incorrectly prepared	
	Standard has gone bad (If	Check the condition of
	there is a signal in the sample	stored standard
	wells)	
	Assay was conducted from an	Reagents allows to come to
	incorrect starting point	20 - 30 °C before performing
		assay
Too much signal-whole plate	Insufficient washing-unbound	• Increase number of washes
turned uniformly blue	Streptavidin-HRP remaining	Carefully
	Too much Streptavidin-HRP	Check dilution
	Plate sealer or reservoir	Use fresh plate sealer and
	reused, resulting in presence of	reagent reservoir for each
	residual Streptavidin-HRP	step
Standard curve achieved but	Plate not developed long	Increase substrate solution
poor discrimination between	enough	incubation time
point	Improper calculation of	Check dilution, make new
	standard curve dilution	standard curve
No signal when a signal is	Sample matrix is masking	More diluted sample
expected, but standard curve	detection	Recommended
looks fine		
Samples are reading too high,	Samples contain protein levels	Dilute samples and run
but standard curve is fine	above assay range	Again
Edge effect	Uneven temperature around	Avoid incubating plate in
	work surface	areas where environmental
		conditions vary
		Use plate sealer



XVI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

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XVII. NOTES