Human MCAF / MCP-1 ELISA Kit

For the Quantitative Determination of Human Monocyte Chemotactic and Activating Factor (MCAF) / Monocyte Chemotactic Protein-1 (MCP-1) Concentrations in Serum, Plasma, Cell Culture Supernatant, and Other Biological Fluids.

Catalogue Number:

EL10009-2 *(192 tests × 1)*
EL10009-6 *(192 tests × 3)*

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

TABLE OF CONTENTS

INTENDED USE

This Human MCAF ELISA kit is to be used for the *in vitro* quantitative determination of human monocyte chemotactic and activating factor (MCAF) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY and is not to be used in diagnostic or therapeutic procedures.

INTRODUCTION

Monocyte chemotactic and activating factor (MCAF), also known as monocyte chemotactic protein 1 (MCP-1), lymphocyte–derived chemotactic factor (LDCF), and glioma-derived chemotactic factor (GDF), is a recently-identified chemotactic cytokine for monocytes. cDNA cloning and structural analysis has revealed that this 76-amino acid polypeptide with a predicted molecular mass of 8,700 daltons belongs to a family of structurally-related low molecular weight proteins characterised by four conserved cysteine residues designated C-C family or intercrine β family (1,2,3).

MCAF is expressed by various types of cultured cells including monocytes, lymphocytes, fibroblasts, endothelial cells, smooth muscle cells and transformed cell lines upon stimulation with LPS or cytokines such as IL-1, TNF-α, and IFN-γ. Although there exist some minor differences in expression patterns that are observed in some types of cells, almost all agents that induce IL-8 mRNA expression also induce MCAF mRNA expression. Platelet-derived growth factor is a strong inducer of MCAF mRNA in human fibroblasts whereas it failed to induce IL-8 mRNA in human fibroblasts (4). These results suggest that the regulatory mechanism of MCAF gene expression differs from that of the IL-8 gene. In addition to being chemotactic for monocytes, MCAF also activates human monocytes to become cytostatic for several human tumour cell lines (5), release lysosomal enzymes (6), and generate superoxide (6).

MCAF is also expressed *in vivo* by lung epithelial cells in patients with idiopathic pulmonary fibrosis (7), synovial tissues of rheumatoid arthritis (8), or in atheromatous plaques in atherosclerotic lesion (9), suggesting the participation of MCAF in the pathogenesis of these disorders. Furthermore, MCAF has a potent histamine-releasing activity on basophils (10) that indicates an associated effect in allergic inflammations. MCAF expression *in vivo* has been investigated by qualitative methods such as *in situ* hybridization and immunohistochemistry. In order to further clarify and elucidate its participation and relation with various disorders, quantitative analysis of its *in vivo* level is necessary (11, 12).

This MCAF ELISA is a 2.5-hour solid-phase immunoassay readily applicable to measure MCAF levels in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 1600 pg/mL. It has shown no cross-reactivity with various other C-C and C-X-C chemokines IL-8 superfamily proteins. This MCAF ELISA is expected to be effectively used for further investigation into the relationship between MCAF and various diseases.

PRINCIPLE OF THE ASSAY

This MCAF enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for MCAF. Standards or samples are then added to the appropriate microtiter plate wells and incubated. MCAF, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound MCAF and other components of the sample. In order to quantify the amount of MCAF present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific for MCAF is added to each well to "sandwich" the MCAF immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain MCAF and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of MCAF in the samples, this kit contains two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant/ urine testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus MCAF concentration (pg/mL). The concentration of MCAF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	192 tests × 1	***192 tests × 3***

1. **MCAF/MCP-1 MICROTITER PLATE** (Part EL09-1) ***96 wells × 2*** ***96 wells × 6***
   Pre-coated with anti-human MCAF monoclonal antibody.

2. **MCAF/MCP-1 CONJUGATE** (Part EL09-2) ***24 mL*** ***24 mL × 3***
   Anti-human MCAF monoclonal antibody conjugated to horseradish peroxidase with preservative.

3. **MCAF/MCP-1 STANDARD** (Part EL09-3) ***4 vials*** ***4 vials × 3***
   Recombinant human MCAF (3.2 ng/vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT I** (Part EL09-4) ***50 mL*** ***50 mL × 3***
   Animal serum with preservative. *For serum/plasma testing.*

5. **CALIBRATOR DILUENT II** (Part EL09-5) ***50 mL*** ***50 mL × 3***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant/urine testing.*

6. **WASH BUFFER (30X)** (Part 30009) ***60 mL*** ***60 mL × 3***
   30-fold concentrated solution of buffered surfactant.

7. **SUBSTRATE A** (Part EL09-6) ***20 mL*** ***20 mL × 3***
   Buffered solution with H_2O_2.

8. **SUBSTRATE B** (Part 30007) ***20 mL*** ***20 mL × 3***
   Buffered solution with TMB.

9. **STOP SOLUTION** (Part 30008) ***28 mL*** ***28 mL × 3***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL required for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.
12. Crystal formation in the wash buffer (30X) will not affect its quality. Prior to dilution, make sure all crystals are completely dissolved by warming to 37°C.

SAMPLE PREPARATION

1. COLLECTION, HANDLING, AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate.

- Samples should be used immediately. Otherwise, samples must be aliquoted and stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Dissolving the crystals in Wash Buffer (30X) by warming up to 37°C. Add 60 mL of Wash Buffer (30X) and dilute to a final volume of 1800 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (30X) to 29 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

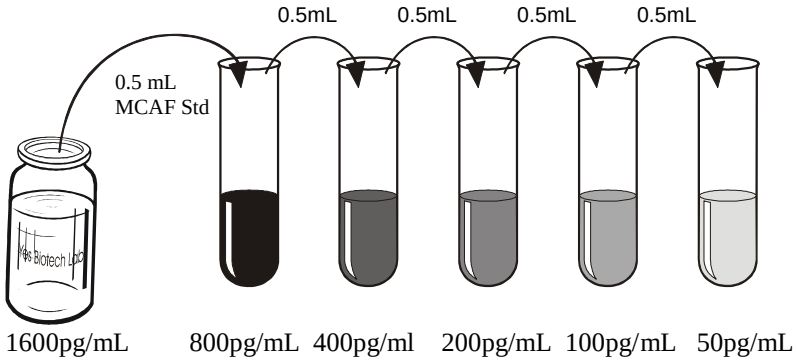
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **MCAF Standard:**

a) Two vials of Standards are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute the MCAF Standard with either 2.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 1600 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The MCAF standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (50 pg/mL to 1600 pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted MCAF Standard will serve as the high standard (1600 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and MCAF Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0 pg/mL**	(S1)	**2A, 2B**	Standard 5 - **400 pg/mL**	(S5)
1C, 1D	Standard 2 - **50 pg/mL**	(S2)	**2C, 2D**	Standard 6 - **800 pg/mL**	(S6)
1E, 1F	Standard 3 - **100 pg/mL**	(S3)	**2E, 2F**	Standard 7 - **1600 pg/mL**	(S7)
1G, 1H	Standard 4 - **200 pg/mL**	(S4)	**2G-12H**	MCAF samples	

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100 µL of Standard or sample to the appropriate well of the antibody pre-coated Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Dispense 100µL of conjugate to each well. Cover and incubate for 1 hour at room temperature.

5. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

6. Repeat wash procedure as described in Step 3.

7. Add 100 μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100 μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of MCAF in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding MCAF concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of MCAF in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding MCAF concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

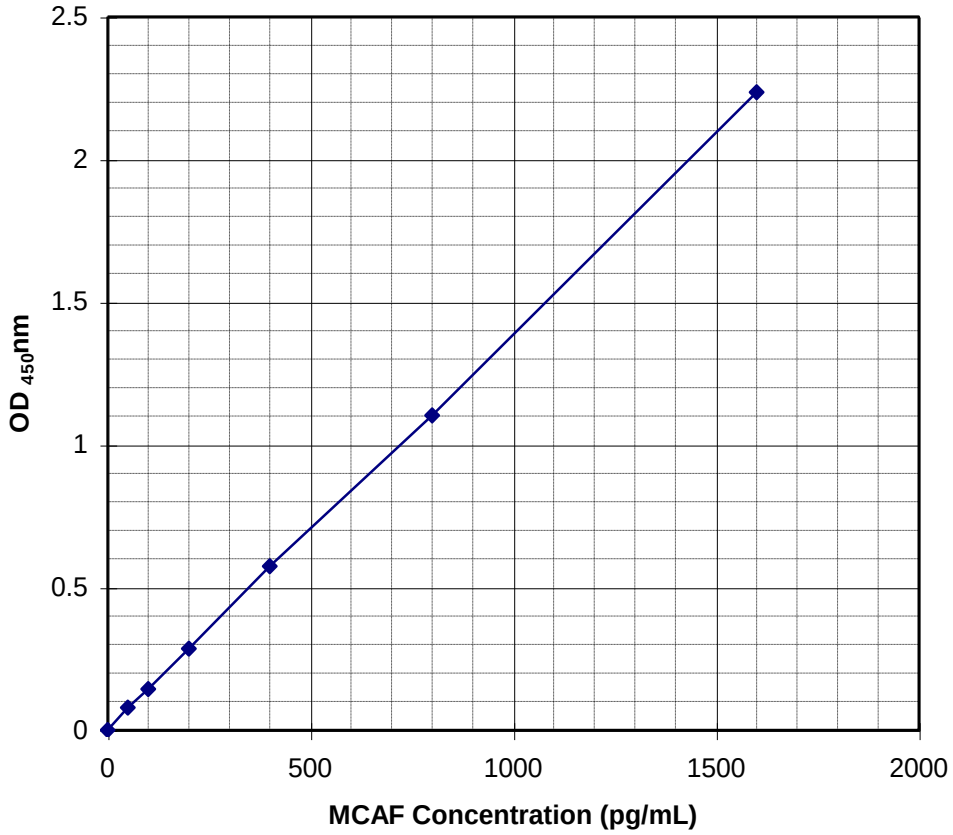
TYPICAL DATA

Results of a typical standard run of a MCAF ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

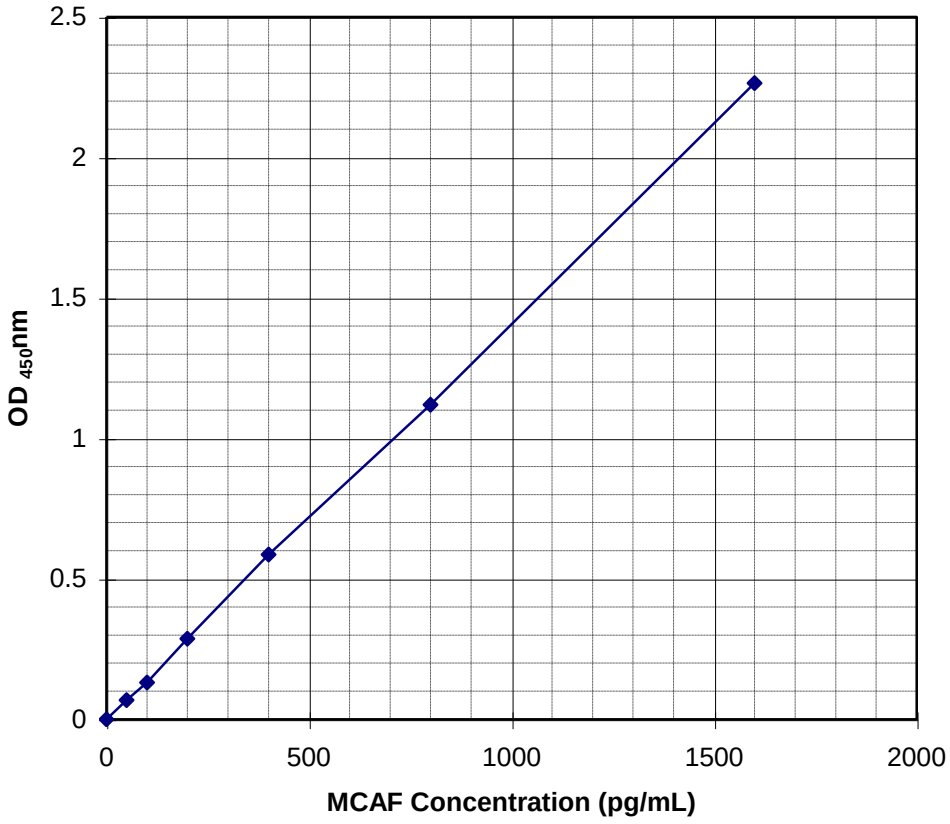
Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.)-(S1)
0	0.029, 0.031	0.030	0
50	0.109, 0.110	0.110	0.079
100	0.171, 0.179	0.175	0.144
200	0.317, 0.315	0.316	0.285
400	0.614, 0.595	0.605	0.574
800	1.149, 1.118	1.134	1.103
1600	2.281, 2.250	2.266	2.235



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) -(S1)
0	0.030, 0.032	0.031	0
50	0.100, 0.098	0.099	0.068
100	0.163, 0.160	0.162	0.131
200	0.316, 0.320	0.318	0.287
400	0.625, 0.610	0.618	0.587
800	1.150, 1.151	1.151	1.120
1600	2.300, 2.290	2.295	2.264



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by using 20 replicates in 1 assay.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	99.20	286.10	942.30
Standard Deviation (pg/mL)	5.71	10.50	31.60
Coefficient of Variation (%)	5.76	3.67	3.35

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were assayed by using replicates on 18 different assays.

	Calibrator Diluent II assay		
Sample	**1**	**2**	**3**
n	18	18	18
Mean (pg/mL)	111.70	326.70	994.40
Standard Deviation (pg/mL)	10.30	32.50	89.40
Coefficient of Variation (%)	9.22	9.95	8.99

3. RECOVERY

The recovery of MCAF within three different levels in various matrices was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture media	104	90 -120
Serum	98	90 - 114
Plasma	98	89 - 112

4. SENSITIVITY

The minimum detectable dose of MCAF was determined by adding two standard deviations to the mean optical density value of the 10 zero-standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose using a standard curve generated with Calibrator Diluent I is *1.5 pg/mL* and using Calibrator Diluent II is *4 pg/mL*.

5. SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human MCAF. This kit exhibits no detectable cross-reactivity with human; EPO, FGF, GM-CSF, IL-8, IL-1ß, MCP-3, M-CSF, RANTES, SAA, TGF-α, TGF-ß, and TNF-α.

6. CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli expressed as a 76 amino-acid polypeptide form of recombinant human MCAF.

7. EXPECTED NORMAL VALUES

Biological samples from apparently healthy, normal individuals were collected and the average MCAF concentration was measured. The average quality in serum/plasma samples (n=30) was 145-350 pg/mL.

REFERENCES

1. Oppenheim, J.J. et al. (1991) *Ann. Rev. Immunol.* 9: 617.
2. Miller, M.D., et al. (1992) *Crit. Rev. Immunol.* 12: 17.
3. Mantovani, A. et al. (1992) *Immunol*. *Today*. 13: 265.
4. Yashimura, T., et al. (1990) *J. Immunol.* 144: 2377.
5. Matsushima, K. et al. (1989) *J. Exp. Med.* 169: 1485.
6. Zachariae, C.O. et al. (1990) *J. Exp. Med.* 171: 2177
7. Antoniades, H.N. et al. (1992) *Proc. Natl. Acad. Sci. USA*. 89: 5371.
8. Koch, A.E. (1992) *J. Clin. Invest*. 90: 772.
9. Yla-Herttuala, S. et al. (1991) Proc, Natl. Acad. Sci. USA. 88:5252.
10. Kuna, P. et al. (1992) *J. Exp. Med.* 175: 489.
11. Peri, G. et al. (1994) *J. Immunol. Methods*. 174: 249.
12. Ida, N. et al. (1994) *Cytokine.* 6: 32.
13. Elisa A. Liehn et al. (2010) A New Monocyte Chemotactic Protein-1/Chemokine CC Motif Ligand-2 Competitor Limiting Neointima Formation and Myocardial Ischemia/Reperfusion Injury in Mice. Journal of the American College of Cardiology. Volume 56, Issue 22, 23 November 2010, Pages 1847–1857

CITATIONS

1. Elisa A. Liehn et al. A New Monocyte Chemotactic Protein-1/Chemokine CC Motif Ligand-2 Competitor Limiting Neointima Formation and Myocardial Ischemia/Reperfusion Injury in Mice. Journal of the American College of Cardiology Volume 56, Issue 22, 23 November 2010, Pages 1847–1857.
2. Margaret J. Burns and Martha B. Furie. Borrelia burgdorferi and Interleukin-1 Promote the Transendothelial Migration of Monocytes In Vitro by Different Mechanisms. Infect Immun. Oct 1998; 66(10): 4875–4883.
3. Sarchielli P et al. Chemokine levels in the jugular venous blood of migraine without aura patients during attacks. Headache. 2004 Nov-Dec;44(10):961-8.
4. Kalayoglu MV et al. Identification of Chlamydia pneumoniae within human choroidal neovascular membranes secondary to age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol. 2005 Nov;243(11):1080-90. Epub 2005 May 21.