

Human MCAF(MCP-1) ELISpot Kit

For the quantitation of single cells releasing human MCAF.

Catalogue Number: SL10009E

96 tests

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 CONTENT

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	3
REAGENTS PROVIDED	3
MATERIALS REQUIRED BUT NOT SUPPLIED	4
PRECAUTIONS	4
SAMPLE PREPARATION	4
ASSAY PROCEDURE	5
REFERENCES	6

INTENDED USE

Human MCAF enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human MCAF.

For laboratory research use only. Not for use in diagnostic procedures.

INTRODUCTION

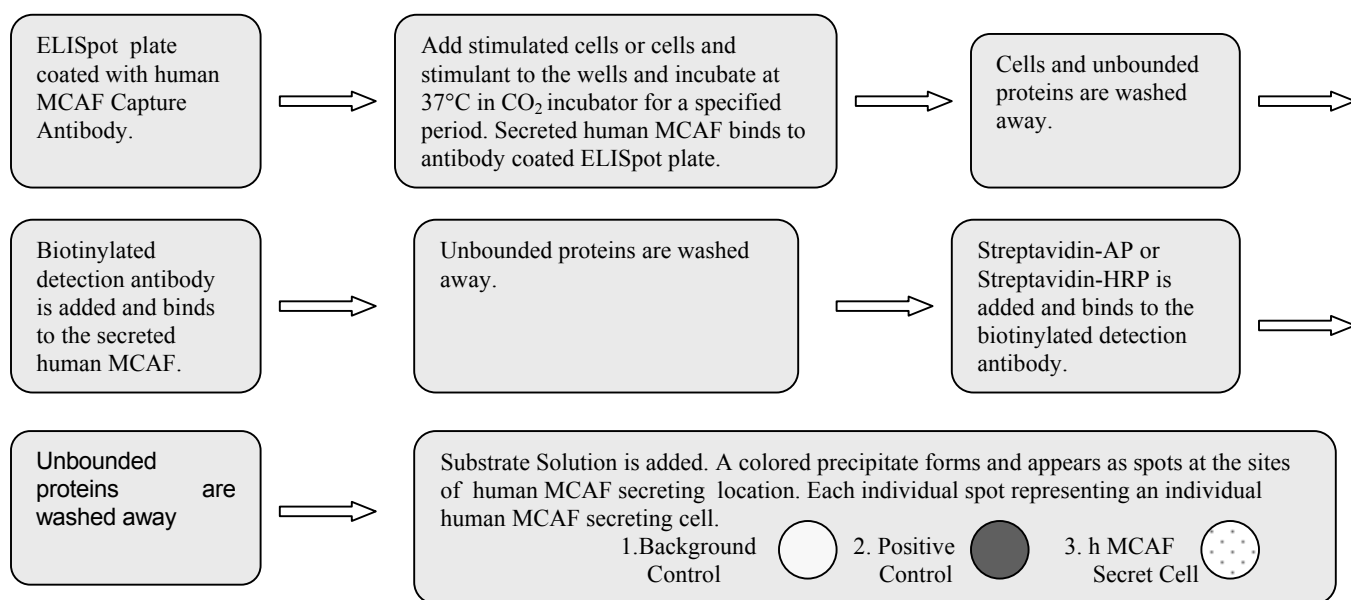
Monocyte chemotactic and activating factor (MCAF), also known as monocyte chemotactic protein 1 (MCP-1), lymphocyte-derived chemotactic factor (LDCF), and glioma-derived chemotactic factor (GDF), is a recently-identified chemotactic cytokine for monocytes. cDNA cloning and structural analysis has revealed 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 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human MCAF.

PRINCIPLES OF THE ASSAY



REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1) ELISpot Plates (1X 96tests, Part SL10009E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human MCAF monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10009E-2)	1 Vial	Lyophilized recombinant human MCAF (2ng/vial)	Reconstitute 1 vial in 250 µL Cell Culture Media before use. Use in 1 hour. The final concentration is 8 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10009E-3)	1 X 60mL	—	Add 1 volume of 20X Wash Buffer Concentrated to 19 volume of deionized water/distilled water. Use in 1 week. Stored at room temperature.
4) Concentrated Human MCAF Detection Antibody (Part SL 10009E-4)	1 Vial	120µL 100 x Concentrated Biotinylated mouse anti-human MCAF monoclonal antibody	Add 1 volume of Human MCAF Concentrated Detection Antibody to 100 volumes of Detection Antibody Diluent (Part SL 10009E-5) before use. Use in 1 month. Stored at 2-8 °C.
5) Detection Antibody Diluent (Part SL 10009E-5)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
6) Concentrated	1 Vial	120µL 100 x	Add 1 volume of Concentrated Streptavidin - AP to

Streptavidin - AP (Part SL 10009E-6)		Concentrated Alkaline Phosphatase labeled Streptavidin.	100 volumes of Streptavidin – AP Diluent (Part SL 10009E-7) before use. Use in 1 month. Stored at 2-8 °C.
7) Streptavidin – AP Diluent (Part SL 10009E-7)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
8) Substrate Solution (Part SL 10009E-8)	1 x 11mL	BCIP/NBT Substrate Solution.	Ready to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Pipettes with disposable tips, bottles, test tubes and racks, graduated cylinders, absorbent paper, and squirt bottle.
2. 37°C CO₂ incubator.
3. Deionized or distilled water.
4. Dissection microscope or ELISpot reader.

PRECAUTIONS

1. Allow kit reagents and materials to reach room temperature (20-25°C) before use.
2. Do not use kit components beyond their expiration date. Do not substitute reagents from one kit lot to another.
3. The toxicity of the Substrate Solution is not currently known, wear gloves to avoid contact with skin. Follow local, state and federal regulations to dispose of used Substrate Solution.
4. If 20 x Wash Buffer Concentrated is stored at lower temperature (2-8 °C), crystals may form which must be dissolved by warming prior to use.
5. When samples are added to the wells, don't let the pipette tips contact the membrane.
6. Don't let the plate dry during the assay.
7. In order to avoid edge effect don't stack plates during cell incubation.
8. Avoid move the plate during cells incubation period.
9. Don't dry the plate at a temperature higher than 37° C.
10. Spots can't be counted accurately until PVDF membranes were completely dry.

SAMPLE PREPARATION

Each researcher should optimize cell separation method, stimulant, stimulation mode and incubation time.

ASSAY PROCEDURE

S7.5(01) hMCAF SL10009E

Aseptic Procedures: Steps 1 to 3 are aseptic procedures. Use sterile buffers and aseptic conditions, use laminar flow hood for procedures.

1. Wash 1 time with Cell Culture Media
Fill each well completely with sterile Cell Culture Media. Don't discard until cells are ready to be plated.
2. Prepare Positive Control
As described in **REAGENT PROVIDED**
3. Add 2 wells positive control, 2 wells negative control (unstimulated cells), 2 wells background control (sterile cell culture media) and MCAF secreting cells with appropriate concentration to each plate, 100 µL/well. Incubate at 37°C CO₂ incubator for 4-48 hours. Each researcher should determine the optimal incubation time based on the characteristics of the cell.

Non-aseptic Procedures: The following steps are non-aseptic procedures.

4. Prepare 1x Wash Buffer, Human MCAF Detection Antibody solution, and Streptavidin – AP solution.
As described in **REAGENT PROVIDED.**
5. Wash the plate 5 times with 1 x Wash Buffer
Decant or aspirate contents of the plate into a waste container. Fill each well completely with 1 x Wash Buffer then decant or aspirate contents of the plate into a waste container. Repeat this procedure 4 more times for a total of 5 washes. After final wash, invert plate, and dry by hitting plate onto absorbent paper slightly.
6. Immediately add 100 µL of Human MCAF Detection Antibody to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
7. Repeat wash procedure as described in step 5. Wash plate 5 times.
8. Immediately add 100 µL of Streptavidin-AP to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
9. Repeat wash procedure as described in step 5. Wash plate 5 times.
10. Immediately add 100 µL of Substrate Solution to each well of the plate. Cover the plate and incubate 5-15 minutes at room temperature (20-25 °C) in dark.
11. Stop the assay
Rinse 5 times with deionized water/distilled water. After final wash, invert plate, and dry by hitting plate onto absorbent paper slightly.
13. Dry plate
Wet plates show higher background than completely dry plates. Remove the plastic underdrain from bottom of the plate. Allow the plate dry for 60-90 min at room temperature, or over night at room temperature, or 15-30 min at 37° C in dark. We recommend dry plate over night at room temperature.
14. Quantify spots using a dissection microscope or ELISpot reader.
15. Dried plate can be stored in sealed plastic bag in dark for 6 months.

REFERENCES

1. Oppenheim, J.J. et al. (1991) *Ann. Rev. Immunol.* 9: 617.
- S7.5(01) hMCAF SL10009E

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. Antoniadou, 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.