Human MCAF (MCP-1) Matched Antibody Pair for ELISpot

Pre-titered capture antibody and biotinylated detection antibody matched pair for the development of enzyme-linked immunospot (ELISpot) assays for the quantitation of single cells releasing human MCAF.

Catalogue Number: SL10009A

Designed for 5 x 96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE

Pre-titered capture antibody and biotinylated detection antibody matched pair for the development of enzyme-linked immunospot (ELISpot) assays for the quantitation of single cells releasing human MCAF.

A recommended assay protocol is provided. The dilutions of capture antibody and detection antibody is determined according to this protocol. The researcher can optimize the dilutions if it is necessary.

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INTRODUCTION

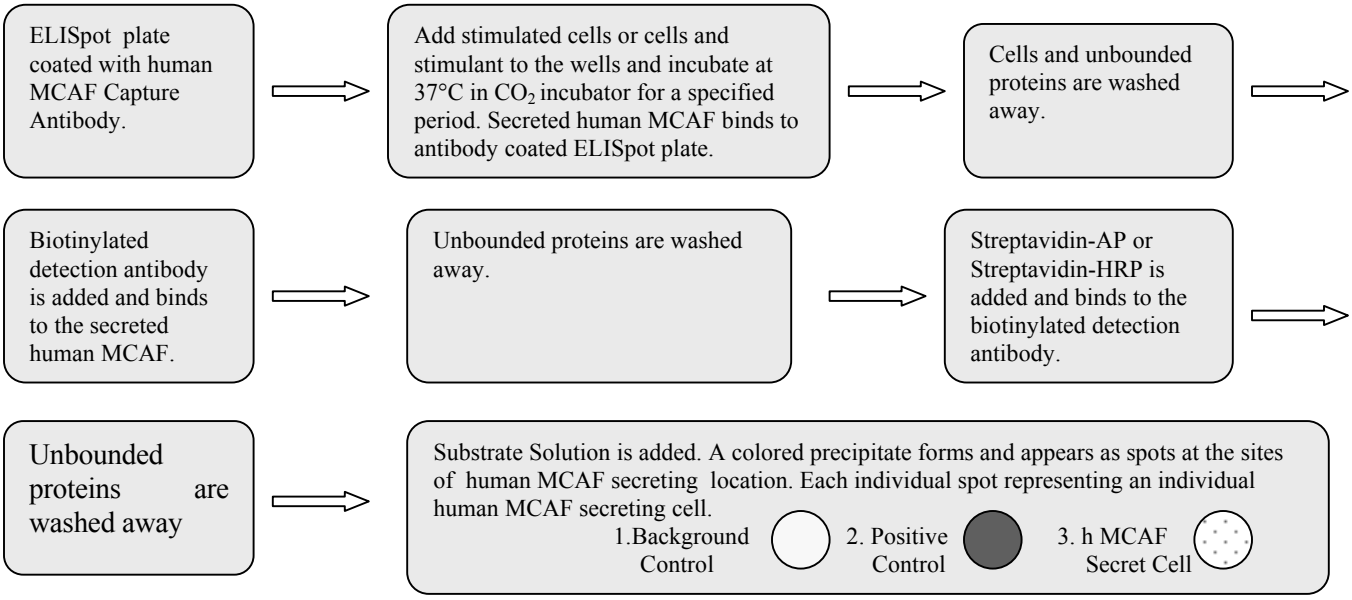
Monocyte chemotactic and activating factor (MCAF), also known as monocyte chemotactic protein 1 (MCP-1), lymphocyte–derived chemotactic factor (LDCF), and glioma-derived chemotactic factor (GDF), is a recently-identified chemotactic cytokine for monocytes. cDNA cloning and structural analysis has revealed 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 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 -20°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1)Concentrated human MCAF Capture Antibody (Part SL10009A-1)	1 Vial	Lyophilized mouse anti-human MCAF monoclonal antibody	Stock Solution: Reconstitute Concentrated Human MCAF Capture Antibody with 0.6 mL PBS. Aliquot if repeated use is expected. The stock solution can be stored frozen (-20˚C to -70˚C) for up to 6 months. Avoid freeze-thaw cycles. Working Solution: When PVDF -bottom Immunospot plates are used, the recommended dilution is 1: 100. Calculate the volume of Capture Antibody Stock Solution needed and dilute to working solution in PBS. Use in 1 hour.
2)Concentrated human MCAF Detection Antibody (Part SL 10009A-2)	1 Vial	Concentrated Biotinylated mouse anti-human MCAF monoclonal antibody	Stock Solution: Reconstitute Concentrated human MCAF Detection Antibody with 0.6 mL Reagent Diluent. Aliquot if repeated use is expected. The stock solution can be stored frozen (-20˚C to -70˚C) for up to 6 months. Avoid freeze-thaw cycles. Working Solution: When PVDF -bottom Immunospot plates are used, the recommended dilution is 1: 100. Calculate the volume of Detection Antibody Stock Solution needed and dilute to working solution in Reagent Diluent. Use in 1 hour.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. **PBS**
   137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2-7.4, 0.2µm filtered.
2. **Wash Buffer**
   0.05% Tween-20 in PBS.
3. **Blocking Buffer**
   1% BSA, 5% Sucrose in PBS, 0.2µm filtered.
4. **Reagent Diluent**
   1% BSA in PBS.
5. **Positive Control (Recommended)**
   Recombinant human MCAF (2ng/vial, Yes Biotech, Catalogue Number SL10009C) or equivalent.
6. **ELISpot Plates**
   PVDF -bottom Immunospot plates or equivalent.
7. **Streptavidin-AP or Streptavidin-HRP**
8. **Substrate Solution**
   8.1 Substrate Solution for Streptavidin-AP color system.
   Yes Biotech BCIP/NBT Substrate Solution for ELISpot (10mL/bottle, Yes Biotech, Catalogue Number SS6006) or equivalent for Streptavidin-AP color system.
   8.2 AEC Substrate Solution for Streptavidin-HRP color system.
   8.2.1 0.1M Phosphate-Citrate Buffer (PH5.0)
   Citric Acid Solution: 9.6g Citric Acid to 500 mL Deionized or Distilled Water.
   Dibasic Sodium Phosphate Solution: 14.2 g Dibasic Sodium Phosphate to 500mL Deionized or Distilled Water.
   Add Dibasic Sodium Phosphate Solution to Citric Acid Solution until the pH to 5.0.
   1:1 Dilute with Deionized or Distilled Water.
   8.2.2 Dissolve 4 mg of AEC (3-amino-9-ethyl-carbazole) in 1 mL of DMF (Dimethyl Formamide).
   8.2.3 Add 14 mL of 0.1M Phosphate-Citrate Buffer (PH5.0)
   8.2.4 Filter through a 0.45 µm filter.
   8.2.5 Just before use, add 10 µL of 30% H_2O_2.
9. **Pipettes with disposable tips, test tubes and racks, graduated cylinders, absorbent paper, and squirt bottle.**
10. **37˚C CO_2 incubator.**
11. **Deionized or Distilled Water.**
12. **Dissection microscope or ELISpot reader.**

PRECAUTIONS

1. Allow kit reagents and materials to reach room temperature (20-25ºC) 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 ºC), 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

Aseptic Procedures: *Steps 1 to 7 are aseptic procedures. Use sterile buffers and aseptic conditions, use laminar flow hood for procedures.*

1. Prepare Human MCAF Capture Antibody Working Solution
   As described in **REAGENT PROVIDED.**
2. Add 100 µL of Human MCAF Capture Antibody Working Solution to each well of the plate. Cover the plate and incubate overnight at 2-8 ˚C.
3. Wash 3 times with PBS
   Decant or aspirate contents of the plate into a waste container. Fill each well completely with PBS then decant or aspirate contents of the plate into a waste container. Repeat this procedure 2 more times for a total of 3 washes. After final wash, invert plate, and dry by tapping plate onto absorbent paper slightly.
4. Blocking
   Immediately add 200 µL of Blocking Buffer to each well of the plate. Cover the plate and incubate 2 hours at 37˚C.
5. Prepare Positive Control
   We recommend adding 2 wells positive control. If Yes Biotech MCAF Positive Control (2ng/vial, Catalogue Number SL10009C) was used, add 250 µL Cell Culture Media to each vial. The final concentration is 8 ng/mL. Use within one hour of reconstituting. The reconstitution can be stored frozen (-20°C) for up to 30 days.
6. Wash 1 time with Cell Culture Media
   Decant or aspirate contents of the plate into a waste container. Fill each well completely with Cell Culture Media. Don't discard until cells are ready to be plated.
7. Decant or aspirate contents of the plate into a waste container, invert plate, and dry by tapping plate onto absorbent paper slightly. Immediately add 100 µL MCAF secreting cells with appropriate concentration to each well. We recommend adding 2 wells positive control, 2 wells negative control (unstimulated cells), and 2 wells background control (sterile cell culture media) in each plate, 100 µL/well. Incubate at 37°C CO_2 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.*

8. Prepare Human MCAF Detection Antibody Working Solution

As described in **REAGENT PROVIDED.**

9. Prepare Streptavidin - AP or Streptavidin-HRP Working Solution
   Each researcher should optimize the concentration of Streptavidin - AP or Streptavidin-HRP Working Solution. Calculate the volume of Streptavidin - AP or Streptavidin-HRP Stock Solution needed and dilute to working solution in Reagent Diluent. Use in 1 hour.
10. Wash the plate 5 times with Wash Buffer
    Decant or aspirate contents of the plate into a waste container. Fill each well completely with 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 tapping plate onto absorbent paper slightly.
11. Immediately add 100 µL of Human MCAF Detection Antibody Working Solution to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
12. Repeat wash procedure as described in step 10. Wash plate 5 times.
13. Immediately add 100 µL of Streptavidin-AP or Streptavidin-HRP Working Solution to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
14. Repeat wash procedure as described in step 10. Wash plate 5 times.
15. 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. Each researcher should optimize the incubate time depending on the plate, reagents or substrate solution used.
16. Stop the assay
    Rinse 5 times with deionized water/distilled water. After final wash, invert plate, and dry by tapping plate onto absorbent paper slightly.
17. Dry plate
    Wet plates show higher background than completely dry plates. Remove the plastic underdrain 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.
18. Quantify spots using a dissection microscope or ELISpot reader.
19. Dried plate can be stored in sealed plastic bag in dark for 6 months.

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