*Your #1 source for antibodies!*

# Human M-CSF ELISpot Kit

For the quantitation of single cells releasing human Macrophage Colony Stimulating Factor (M-CSF).

Catalog # MBS591012

*96 tests*

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

S7.5(00) hM-CSF SL10046E

## TABLE OF CONTENT

## INTENDED USE

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

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

## INTRODUCTION

Human M-CSF, also known as CSF-1, can be either expressed on the cell surface as a membrane-spanning 68-86 kDa chondroitin or secreted as an 80-100 kDa glycoprotein or 130-160 kDa chondroitin sulfate-containing proteoglycan. The biologically active forms of M-CSF are dimeric. The following tissues are known producers of M-CSF: submaxillary gland, lung, spleen, kidney, lymph nodes, brain, liver, testis, ovary, and some human tumors. M-CSF can be synthesized in most cell types including fibroblasts, endothelial cells, bone marrow stromal cells, osteoblasts, thymic epithelial cells, keratinocytes, astrocytes, myoblasts, mesothelial cells, liver parenchymal cells, thyrocytes, and adipocytes. The primary biological activities of M-CSF are related to the survival, proliferation, and differentiation of mononuclear phagocytes. Many conditions are accompanied by elevated M-CSF levels, such as pregnancy, neoplastic disorders of hematopoietic and reproductive systems, preedampsia, chemotherapy with and without autologous bone marrow transplantation, infection, liver disease, hepatic injury, hemophagocytic syndrome, thalassemia, amyloidosis, ischemic heart disease, ovarian cancer, endometrial cancer, breast cancer, and amyloidosis.

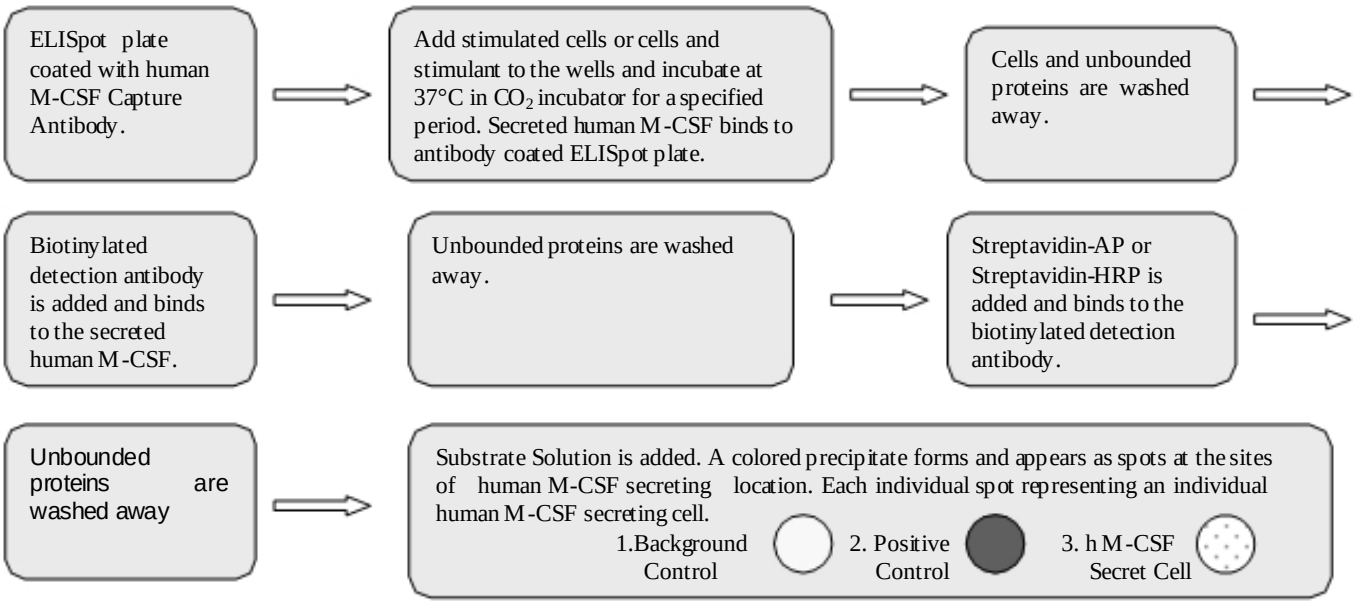
The human M-CSF gene has a length of approximately 20 kb and contains ten exons. The gene has recently been assigned to chromosome 1p13-p212, which is in the vicinity of the amylase genes. IL-2 induces gene expression of M-CSF in human blood-derived monocytes, and NF-kappa B is involved in transcriptional regulation of the M-CSF gene.

A genetic variation of the M-CSF gene exists in humans and appears to substantially increase atherosclerosis risk among smokers.

The biological activities of M-CSF are mediated by a receptor of 165 kDa in length encoded by a gene that maps to human chromosome 5q33. The M-CSF receptor is identical with the proto-oncogene *fms*, and has been subsequently named CD115.

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human M-CSF.

## 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 SL10046E-1) | 1X 96tests | PVDF - bottom Immunospot plates pre-coated with mouse anti-human M-CSF monoclonal antibody. | Unpacked before use |
| 2) Positive Control (Part SL10046E-2) | 1 Vial | Lyophilized recombinant human M-CSF (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 SL10046E-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) Human M-CSF Detection Antibody (Part SL 10046E-4) | 1 x 11mL | Biotinylated mouse anti-human M-CSF monoclonal antibody | Ready to use. |
| 5) Concentrated Streptavidin - AP (Part SL 10046E-5) | 1 Vial | 120µL 100 x Concentrated Alkaline Phosphatase | Add 1 volume of Concentrated Streptavidin - AP to 100 volumes of Streptavidin - AP Diluent (Part SL 10009E-7) before use. Use in 1 month. Stored at 2-8 °C. |

| | | | |
|---|---|---|---|
| | | labeled Streptavidin. | |
| 6) Streptavidin - AP Diluent (Part SL 10046E-6) | 1 x 11mL | Protein with buffer and preservative. | Ready to use. |
| 7) Substrate Solution (Part SL 10046E-7) | 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℃ $CO_2$ incubator.
3. Deionized or distilled water.
4. 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 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 M-CSF secreting cells with appropriate concentration to 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.*

4. Prepare 1x Wash Buffer 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 M-CSF 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. Bartocci, A., et al. (1986). J. Exp. Med. 164, 956-961.
2 Borrello, M. A., et al. (1999). J. Immunol. 163, 3605-3611.
1. Brach MA et al (1993) J Immunol. 150, 5535-43.
2. Cebon. J., et al. (1994). Br. J. Haematol. 86, 265-274.
3. Frangogiannis, N. G., et al. (1998). J. Mol. Cell. Cardiol. 30, 2567-2576.
4. Grieg. A., Roth. P. (1995). Infect Immun. 63, 1595-1597.
5. Hayashi, M., et al. (1996). Blood 88, 4426-4428.
6. Itoh. Y., et al. (1994). J. Hepatol. 21, 527-535.
7. Itoh. Y. , et al. (1997). J. Hepatol. 26, 244-252.
8. Itoh. Y., et al. (1999). Liver 19, 97-103.
9. Kacinski. B. M., (1995). Ann. Med. 27, 79-85.
10. Katano, K., et al. (1997). Am. J. Reprod Immunol. 38, 1-5.
11. Kimura, F., et al. (1992). Int. J. Hematol. 55, 147-155.
12. Levine, J. A., et al. (1998). J. Clin. Invest. 101,1557-1564.
13. Matsumura. M., et al. (1999). Life Sci. 65, L129-135.
14. Petros. W. P., et al. (1994). Exp. Hematol. 22, 582-586.
15. Price, L. K. H., et al. (1992). J. Biol. Chem. 267, 2190-2199.
16. Rabinowitz. J., et al. (1993). Blood 81, 2452-2459.
17. Roth. P., et al. (1997). J. Immunol. 158, 3874-3880.
18. Rysava. R., et al. (1999). Biochem. Mol. Biol. Int. 47, 845-850.
19. Scholl, S. M., et al. (1996). Breast Cancer Res. Treat. 39, 275-283.
20. Shirono. K., et al. (1995). J. Haematol. 55, 240-244.
21. Stanley, E. R. (1994). The Cytoking Handbook, (ed. A.W. Thomson), Colony stimulating factor-1 (macrophage colony stimulating factor) pp. 387-418 Academic Press, San Diego.
22. Stanley, E. R. (1998). Encyclopedia of Immunology, (ed. P. J. Delves and I. M. Roitt), Macrophage colony stimulating factor CSF-1 pp. 1650-1654 Academic Press, Orlando.
23. Suzu. S., et al. (1992). J. Biol. Chem. 267, 4345-4348.
24. Tashiro, H., et al. (1997). Coron. Artery Dis. 8, 143-147.
25. Wiener, E., et al. (1996). Eur. J. Haematol. 57, 364-369.
26. Tashiro, H., et al. (1997). Coron. Artery Dis. 8, 143-147.
27. Wiener, E., et al. (1996). Eur. J. Haematol. 57, 364-369.