MSD® 96-Well MULTI-ARRAY® and MULTI-SPOT®

Human Macrophage Colony Stimulating Factor (hM-CSF) Ultrasensitive Assay

Summary

This assay measures Human Macrophage Colony Stimulating Factor (M-CSF) in a 96-well MULTI-ARRAY or MULTI-SPOT plate. The assays employ a sandwich immunoassay format where Capture Antibodies are coated in a single spot, or in a patterned array, on the bottom of the wells of a MULTI-ARRAY or MULTI-SPOT (Figure 1) plate. This product insert outlines an assay protocol recommended for serum and plasma samples. Other complex matrices and sample types may be tried using this protocol as a starting point.

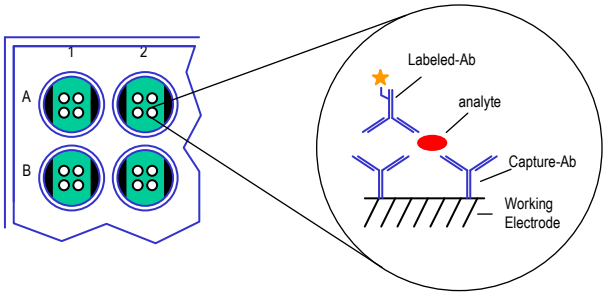

Figure 1. M-CSF Capture Antibody is pre-coated on a Single Spot MULTI-ARRAY plate or on specific spots of a 4-Spot MSD MULTI-SPOT plate. Calibrator solutions and samples are incubated in the plate, and M-CSF binds to corresponding Capture Antibody spot. M-CSF is detected using an M-CSF-specific Detection Antibody labeled with MSD SULFO-TAG™ Reagent.

Reagents Provided

Storage

Detection Antibody	Anti hM-CSF antibody labeled with MSD SULFO-TAG Reagent. Detection Antibody is provided as a 50X stock solution.	2-8 °C
M-CSF Calibrator	Provided at 1 μg/mL.	≤-70 °C
Diluent 2	Protein and animal derived material medium for assay diluent and dilution of Calibrators	≤-10 °C
Diluent 3	Contains blocking and stabilizing agents.	≤-10 °C
Read Buffer T	4X Read Buffer T with surfactant	RT
MULTI-ARRAY or MULTI-SPOT Plate	96-well MSD plate spotted with specific anti M-CSF Capture Antibodies	2-8 °C

Required Materials and Equipment – not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate Buffered Saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of accurately dispensing 10 to 150 μL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

Sample Preparation

This section provides a general guide for the preparation of various clinical sample types for use in MSD assays. Safe laboratory practices and personal protective equipment such as gloves, lab coat, and safety glasses should be used at all times when handling samples. All samples of a potentially infectious or hazardous origin should be handled in the manner outlined by the Center for Disease Control and the Occupational Health and Safety Administration for blood-borne pathogens and Human and animal-source materials.

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Some analytes in this matrix are extremely sensitive to multiple freeze/thaw cycles and the ability to detect these analytes may decrease following the first round of thawing. For normal serum or plasma samples, no dilution is typically required. Serum or plasma with higher-than-normal levels of analyte may require some dilution of the sample.

Reagent Preparation for Serum and Plasma Assay Protocol

Bring all reagents to room temperature.

Prepare Calibrator and Control Solutions:
Dilute Calibrator in Diluent 2. The calibration curve preparation instructions listed below will generate a standard curve from 10000 pg/mL to 2.4 pg/mL. The curve should be adjusted as necessary to provide the proper range for test samples.

Prepare Calibration Curve:

10000 pg/mL:	Add 10 µL of the 1 µg/mL stock solution to 990 µL of Diluent 2. Use this high Calibrator to prepare the standard curve (10000 pg/mL -0 pg/mL) following a 1:4 dilution series (as shown below).
2500 pg/mL:	Add 50 µL of 10,000 pg/mL high Calibrator (10000 pg/mL) to 150 µL of Diluent 2.
625 pg/mL:	Add 50 µL of 2500 pg/mL Calibrator to 150 µL of Diluent 2.
156 pg mL:	Add 50 µL of 625 pg/mL Calibrator to 150 µL of Diluent 2.
39 pg/mL:	Add 50 µL of 156 pg/mL Calibrator to 150 µL of Diluent 2.
9.8 pg/mL:	Add 50 µL of 39 pg/mL Calibrator to 150 µL of Diluent 2.
2.4 pg/mL:	Add 50 µL of 9.8 pg/mL Calibrator to 150 µL of Diluent 2.
0 pg/mL:	150 µL of Diluent 2

Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces.

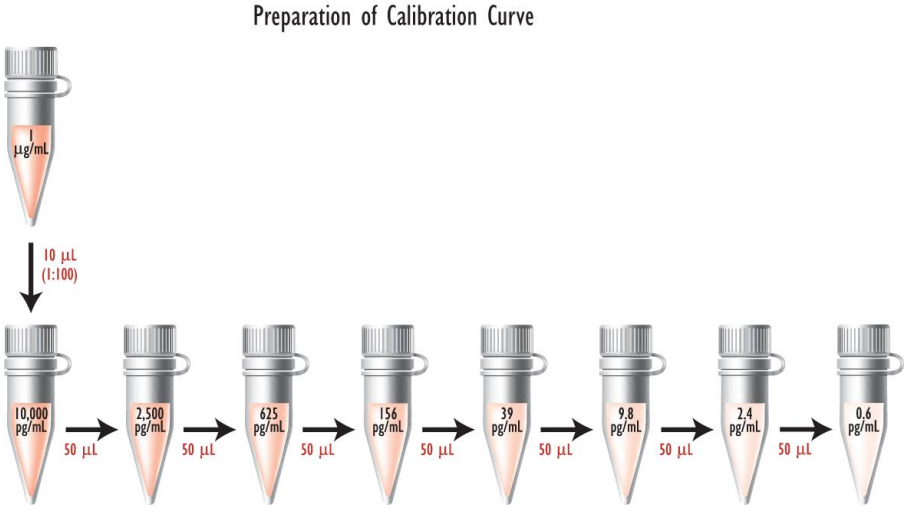

Figure 2. Calibration curve preparation from stock solution (1 µg/mL).

Prepare Detection Antibody Solution:

Detection Antibody solutions should be kept in the dark as some antibodies may be light sensitive. The Detection Antibody is provided at a concentration of 50X. The working Detection Antibody solution should be prepared at 1X. For each plate used, dilute a 60 µL aliquot of the stock Detection Antibody into 2.94 mL of Diluent 3.

***Prepare Read Buffer*:**

The Read Buffer should be diluted 1:2 in deionized water to make a final concentration of 2X Read Buffer T. Add 12.5 mL of stock Read Buffer T (4X) to 12.5 mL of deionized water for each plate.

Protocol

Human Serum and Plasma Assay Protocol

1. ***Addition of Diluent 2:*** Dispense 25 µL of Diluent 2 into each well. Pipette to the bottom of the plate so as to allow the fluid to cover the entire bottom of the well. A slight tap may be necessary to allow the fluid to settle to the bottom. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (300-1000 rpm) at room temperature.

2. ***Addition of Sample or Calibrator:*** Dispense 25 µL of each Calibrator or Sample Solution into a separate well of the MSD plate. Figure 3 illustrates one plate arrangement of Calibrator solutions that can be used to evaluate samples. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

3. ***Wash and Addition of Detection Antibody Solution:*** Wash the plate 3X with PBS + 0.05% Tween-20. Dispense 25 µL of the 1X working Detection Antibody solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300-1000 rpm) at room temperature.

4. ***Wash and Read:*** Wash the plate 3X with PBS + 0.05% Tween-20. Add 150 µL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR® Imager. Plates may be read immediately after addition of Read Buffer. Note: Bubbles in the fluid will interfere with reliable reading of the MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2500 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	625 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	156 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	39 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	9.8 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	2.4 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	0.6 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	0 pg/mL		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample

Figure 3. Suggested plate setup for calibrating MSD M-CSF Assay Kits. Columns 1-2 contain a standard curve in duplicate. The remaining wells are available for samples. An alternative setup that contains a larger dynamic range for the standard curve can be prepared by running Calibrators in a 12-point titration in duplicate across the top or bottom of the plate. The concentrations of Calibrators run may be adjusted depending on the desired dynamic range for the experiment. The data from this calibration curve can be analyzed using any standard data analysis packages.

Additional Notes

1. ***Sample Matrices:*** This M-CSF assay has been found to work well in a range of samples, including serum, heparin plasma, and EDTA plasma samples. Sample dilution and/or spike recovery studies in the sample matrix of interest should be carried out to verify acceptable performance in the matrix.

2. ***Combining the Sample and Detection Antibody Addition Steps:*** The protocol described above calls for incubating the sample in the wells prior to the addition of the Detection Antibody (sequential incubations). This procedure is used because in some selected assays using a polyclonal Detection Antibody, the Detection Antibody itself may include antibodies that compete with the immobilized Capture Antibody. In some assays, however, MSD has found that the sample and Detection Antibodies may be added concurrently (simultaneous incubation) with little or no loss in performance.

Topics of Interest

1. ***Background signal and negative signals:*** The output signal produced by electrochemiluminescence assays is in units of counts of light measured by a charge-coupled device (CCD) camera or photodiode. As with any measurement technique, there is a certain amount of normal variation in this signal (instrument noise) which sets the threshold for the lowest levels of signal that can be measured (noise floor). This variation is different depending upon the size of the working electrode with typical values of about 10 counts for 96-well small spot and 96-well 4-spot plates, 15 counts for 96-well 7-spot plates, and 30 counts for 96-well 10-spot plates. When the background signal of an assay approaches the noise floor (i.e. the mean signal of negative controls or sample blanks is close to zero), it is possible to observe negative counts for some wells.

2. ***Signal Levels:*** The camera system is linear over nearly a 6 log-dynamic range. The highest achievable signals on the SECTOR Imager 6000 and 2400 are between 1.0 and 2.0 million counts. If the signals from the highest point on the calibration curve are not approaching 1.0 million counts, the high end of the calibration curve may be extended. The lowest observed signals using the Read Buffer T (2X) are between 10 and 50. Negative signal values may occur due to instrument noise, omission or usage of the incorrect Read Buffer, or incorrect amount of detection antibody.

3. ***Fitting methods:*** To utilize the quantitative value of electrochemiluminescent detection, a titration curve is produced using a known standard. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. MSD's assays have a wide dynamic range (typically 3-5 logs) which allows accurate quantitation in many samples without the need to dilute prior to running the assay. MSD recommends using software to fit the data that utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve. An alternative analysis approach is to subtract the background signal from all data points, and then use a linear model to fit the data. The disadvantage of this approach is that a skewed calibration curve may be created if the background signal used for subtraction is not an appropriate indicator of background signal over the complete curve. Also, negative numbers may be produced if background-corrected signal values are less than the instrument noise observed in signals at the low end of the curve.

4. ***Antibody pairs and cross-reactivity:*** The Capture and Detection Antibody pairs used in MSD assays have been selected by an optimization process that is designed to minimize cross-reactivity with other assays.

5. ***Reverse pipetting:*** Most manual hand pipettes have two plunger positions for pipetting liquids. The first position is calibrated to allow aspiration and dispensing of user-specified amounts of liquid and the second (blow-out) position enables the user to expel any residual liquid after the pipette has been pushed to the first position. When a pipette is used to dispense liquid by moving the plunger to the first position followed by the second (blow-out) position, bubbles may be created in the dispensed liquid. The reverse pipetting technique is designed to allow precise pipetting while avoiding the creation of bubbles. The technique is to push the pipette plunger past the first position to the second position prior to aspirating liquid into the tip, thereby aspirating slightly more liquid than the desired volume (overdraw). In order to dispense the liquid from the tip, the pipette plunger is pushed to the first position only. This allows precise dispensing without the introduction of bubbles. When using the reverse pipetting technique, it is important not to overdraw excess liquid into the pipette mechanism.

MSD® 96-Well MULTI-ARRAY® and MULTI-SPOT®

hM-CSF Ultrasensitive Assay for Serum and Plasma Samples

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the MSD Human M-CSF Assay.

STEP 1: **Sample and Reagent Preparation**

Samples may not require dilution prior to use in the assay.
Bring appropriate diluents and plates to room temperature and thaw Calibrators on ice. Store Detection Antibody at 4 °C; shield from light.
Prepare Calibrator solutions and calibration curve.

- The Calibrators should be diluted in Diluent 2
- Use the 1 μg/mL Calibrator stock to prepare an 8-point calibration curve of 10000, 2500, 625, 156, 39, 9.8, 2.4 and 0 pg/mL. The calibration curve can be modified as necessary to meet specific assay requirements.

Prepare Detection Antibody solution by diluting the Detection Antibody to 1 μg/mL in 3.0 mL of Diluent 3 (per plate). Keep this reagent in the dark.
Prepare 25 mL of 2X Read Buffer T by diluting 4X MSD Read Buffer T 1:2 with deionized water.
Continue with assay using appropriate protocol below.

STEP 2: **Add Diluent 2**

Dispense 25 μL/well Diluent 2.
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

STEP 3: **Add Sample or Calibrator**

Dispense 25 μL/well Calibrator or Sample.
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

STEP 4: **Wash and Add Detection Antibody**

Wash plate 3X with PBS-0.05% Tween-20.
Dispense 25 μL/well 1X Detection Antibody solution.
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

STEP 5: **Wash and Read Plate**

Wash plate 3X with PBS-0.05% Tween-20.
Dispense 150 μL/well 2X Read Buffer T.
Analyze plate on SECTOR Imager.

1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H