Human M-CSF ELISA Kit

For the quantitative determination of human Macrophage Colony Stimulating Factor (M-CSF) concentrations in serum, plasma and cell culture supernatant

Catalogue Number: EL10046

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (02) M-CSF

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INTENDED USE

This Human M-CSF ELISA Kit is to be used for the *in vitro* quantitative determination of human Macrophage Colony Stimulating Factor (M-CSF) concentrations in serum, plasma and cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic 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, pre-edampsia, 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 M-CSF ELISA is a ready-to-use 4 hours solid phase immunoassay readily capable of measuring M-CSF levels in serum, plasma and cell culture supernatant in the range of 0 to 4000 pg/mL. This assay has shown no cross-reactivity with other cytokines tested, and is expected to be used effectively for further investigations into the relationship between M-CSF and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This M-CSF 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 to M-CSF. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for M-CSF and incubated. If present, M-CSF will bind and become immobilized by the antibody pre-coated on the wells and then become “sandwiched” by biotin conjugate. The microtiter plate wells are thoroughly washed to remove unbound M-CSF and other components of the sample. In order to quantitatively determine the amount of M-CSF present in the sample, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits, each having a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin 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 M-CSF, biotin-conjugated antibody, and enzyme-conjugated Avidin 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 M-CSF in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, 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 M-CSF concentration (pg/mL). The concentration of M-CSF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF APPLICATION

- The Human M-CSF ELISA kit is not for use in clinical diagnostic procedures, and for laboratory use only.
- Although all manufacturing precautions have been exercised to ensure that this product will be suitable for use with all validated sample types as designated in the product insert, the possibility of interference cannot be excluded due to the variety of proteins that may exist within the sample.
- The Calibrator Diluent selected for the standard curve should be consistent with the assay samples. If the values generated by the samples are greater than the uppermost standard, the samples dilution should be adjusted with the appropriate Calibrator Diluent and the assay should be repeated.

REAGENTS PROVIDED

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

	96 tests

1. **M-CSF MICROTITER PLATE** (Part EL46-1) ***96 wells***
   Pre-coated with anti-human M-CSF monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part EL46-2) ***6 mL***
   Anti-human M-CSF polyclonal antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part EL46-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **M-CSF STANDARD** (Part EL46-4) ***2 vials***
   Recombinant human M-CSF (8000pg/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL46-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL46-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL46-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    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 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. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

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.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, cell culture supernatant, and urine samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must 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):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

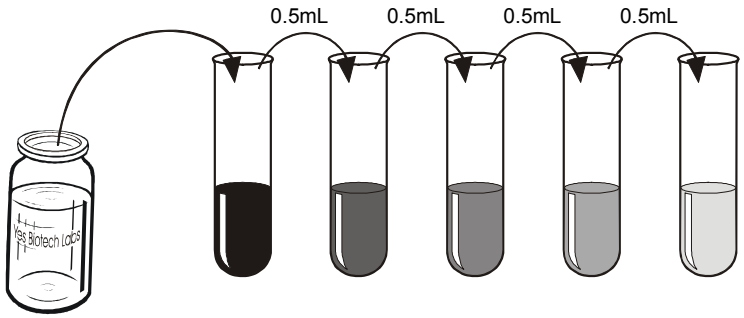
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
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. **M-CSF Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute M-CSF Standard with 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 4000 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 M-CSF 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, as described below, within the range of this assay (0 to 4000 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 M-CSF stock solution will serve as the high standard (4000 pg/mL) and the Calibrator Diluent will serve as the zero-standard (0 pg/mL).



M-CSF Standard 4,000pg/ml	2,000pg/ml	1,000pg/ml	500pg/ml	250pg/ml	125pg/ml

ASSAY PROCEDURE

1. Prepare Wash Buffer and M-CSF 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 and Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0pg/mL**	(S1)	**2A, 2B**	Standard 5 - **1000pg/mL**	(S6)
1C, 1D	Standard 2–**125pg/mL**	(S2)	**2C, 2D**	Standard 6 - **2000pg/mL**	(S7)
1E, 1F	Standard 3- **250pg/mL**	(S3)	**2E, 2F**	Standard 7- **4000pg/mL**	(S7)
1G, 1H	Standard4– **500pg/mL**	(S4)	**3A-12H**	**M-CSF** 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 50 µL of Anti M-CSF biotin conjugate to the antibody pre-coated Microtiter Plate
3. Add 100 µL of Standard or sample to the appropriate wells. Mix well. Cover and Incubate for 2 hours at room temperature.
4. 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.
5. Add 100 µL Avidin Conjugate to each well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).
7. Repeat wash procedure as described in Step 4.
8. Add 100 µL Substrate Solution into each well. Cover and Incubate for 20 minutes at room temperature.
9. Add 100 µL Stop Solution to each well. Mix by gently tapping the plate.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of M-CSF 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 M-CSF 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.

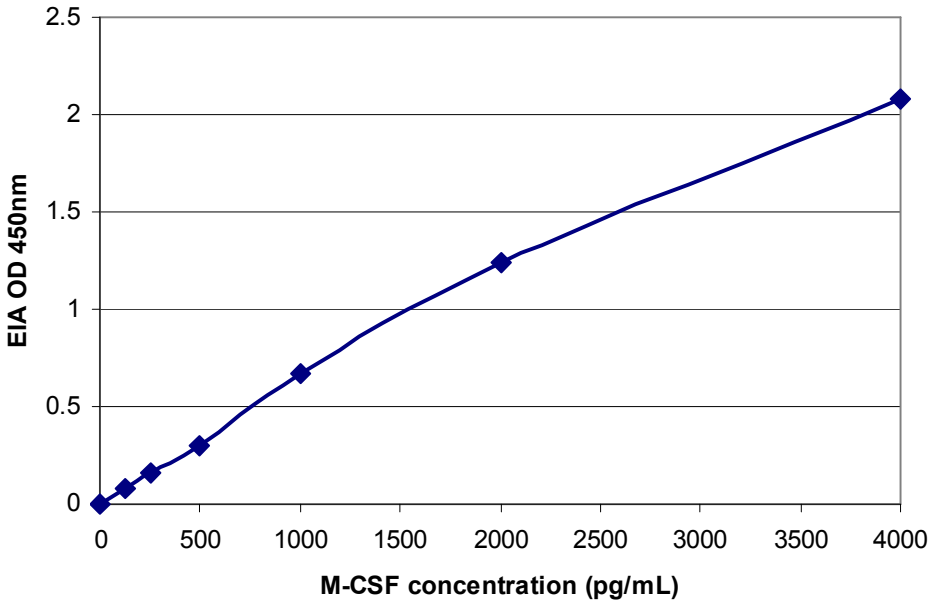
2. To determine the amount of M-CSF in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding M-CSF concentration. If samples generate values higher than the 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 M-CSF 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 their own results.

EXAMPLE

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.056, 0.056	0.056	0
125	0.136, 0.137	0.137	0.081
250	0.222, 0.210	0.216	0.160
500	0.359, 0.362	0.361	0.305
1000	0.741, 0.716	0.729	0.673
2000	1.344, 1.249	1.297	1.241
4000	2.177, 2.090	2.131	2.075



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, 3 different sera were assayed by using 10 replicates in 1 assay.

Sample	**1**	**2**	**3**
n	10	10	10
Mean (pg/mL)	2001.9	1003.3	41.9
Standard Deviation (pg/mL)	135.5	42.8	2.23
Coefficient of Variation (%)	*6.77*	*4.27*	*5.32*

2. INTER-ASSAY PRECISION

To determine between-run precision, 3 different sera were assayed by using replicates on 20 different assays.

Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	2029.7	984.3	36.8
Standard Deviation (pg/mL)	125.6	48.5	2.13
Coefficient of Variation (%)	*6.19*	*4.93*	*5.8*

3. RECOVERY

By employing five samples, the recovery of M-CSF was evaluated with 7 different amounts of M-CSF throughout the range of the assay. All samples were mixed and assayed in duplicate.

Sample Type	***Average Recovery (%)***	***Range (%)***
Cell Culture Media	98	84-110
Serum	97	90-100
EDTA plasma	91	86-98
Heparin plasma	95	89-97
Citrate plasma	91	81-94

4. SENSITIVITY

The minimum detectable quantities of human M-CSF as observed by the standard curve generated for both Calibrator Diluent I and Calibrator Diluent II are 15 pg/mL and 37pg/mL respectively. The two standard deviations above the mean optical density of the 20 replicates of the zero standard were defined as the minimum detectable quantities.

5. SPECIFICITY

This sandwich ELISA can detect both natural and recombinant human M-CSF. This kit exhibits no significant cross-reactivity with human IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-15, IL-16, TNF-α, TGF-β, and TNF-β.

6. CALIBRATION

This immunoassay is calibrated against NIBSC/WHO First International Standard for M-CSF, Code 89/512.

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