

HUMAN M-CSF/CSF1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN M-CSF CONCENTRATIONS IN SERUM AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN M-CSF/CSF1 ELISA KIT
Catalog No.	SK00140-02
Lot No.:	
Formulation	96 T
Standard range	20 – 1280 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, Plasma
Specificity	Human M-CSF
Calibration	Human M-CSF Rec. (HEK293)
Intra-assay Precision	2 - 4%
Inter-assay Precision	4 - 8%
Storage	2 – 8°C for 6 months. More detail check page 2
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human M-CSF/CSF1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human M-CSF from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human M-CSF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human M-CSF samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human DLL4. The capture antibody can bind to the human DLL4 in the standard and samples. After washing the plate of any unbound substances, another monoclonal antibody-HRP conjugate against human DLL4 is added to the wells. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human DLL4 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
M-CSF Microplate – 96 well microplate coated with a monoclonal antibody specific for human M-CSF.	140-02-01	1 plate
M-CSF Standard – 1280 pg/vial of lyophilized recombinant human DLL4.	140-02-02	1 vial
Detection Antibody-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of monoclonal antibody conjugated to HRP against human DLL4.	140-02-03	1 vial
Dilution Buffer - 45 mL of buffered solution with preservative.	DB10	1 bottle
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution - 11 mL of 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 6 months. For longer storage up to 10 months, unopened Standard, Positive Control and Dilution Buffer (DB10) should be stored at -20°C or -70°C. **Detection Antibody-HRP Conjugate and TMB substrate solution should be stored only at 2 ~ 8°C.** Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

M-CSF Standard - Reconstitute the M-CSF standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1280 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **1280 pg/mL** standard serves as the high standard. The

Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1280 pg/ml
# 1	250 μl of stock	250 μl	640 pg/ml
# 2	250 μl of 1	250 μl	320 pg/ml
# 3	250 μl of 2	250 μl	160 pg/ml
# 4	250 μl of 3	250 μl	80 pg/ml
# 5	250 μl of 4	250 μl	40 pg/ml
# 6	250 μl of 5	250 μl	20 pg/ml

Detection Antibody-HRP Conjugate - Pipette 9.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 μL of 100-fold concentrated stock solution to prepare working solution (**protect from light**). **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 μl per well of Dilution Buffer to Blank wells.
3. Add 100 μl per well of standard dilutions from #6 to #S (reverse order of serial dilution), positive control or samples. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (200 rpm).
4. Aspirate wells and wash 4 times with 300 μl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
5. Add 100 μl per well of 1x Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate at room temperature for 1 hour on microplate shaker (350 rpm). **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of Substrate Solution to each well. Incubate for 15-19 minutes on microplate shaker at room temperature. **Protect from light.**

8. Add 100 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human M-CSF	100%
Rat GM-CSF	0
Human CSF1R	0

TYPICAL DATA

This standard curve data is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.061)
20	0.031
40	0.072
80	0.136
160	0.267
320	0.529
640	1.001
1280	1.977

- Lot No.:

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 μ l of standard dilutions, samples or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 μ l per well 1x Detection Antibody-HRP working solution to each well. Incubate 1 hour on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 μ l TMB Substrate Solution to each well. Incubate 15-19 min on the plate shaker at RT. Protect from light.
↓
Add 100 μ l Stop Solution to each well. Read at 450nm within 5 min.