Version 10-04/24

User's Manual

Mouse Colony Stimulating Factor 1 (Macrophage), Csf1 ELISA Kit

REF **CKERS-CSF-052M**

Σ **15 plates, 45 plates**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Mouse M-CSF ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant M-CSF in a sandwich ELISA format within the range of 32 -1,000 pg/mL. Using the ELISA protocol described below, this kit provides sufficient reagents to assay M-CSF in approximately 1,500 ELISA plate wells.

General Description

Macrophage colony-stimulating factor, or M-CSF, is a secreted cytokine which influences hemopoietic stem cells to differentiate into macrophages or other related cell types. M-CSF is a key regulator of cellular proliferation, differentiation, and survival of blood monocytes, tissue macrophages and their progenitor cells. It enhances cytotoxicity, superoxide production, phagocytosis, chemotaxis, and secondary cytokine production in monocytes and macrophages. It binds to the Colony stimulating factor 1 receptor.

Materials Required But Not Supplied

RECOMMENDED SOLUTIONS:

PBS: Dilute 10xPBS to 1xPBS, pH 7.2 in sterile water.

Wash Buffer: 0.05% Tween-20 in PBS

Block Buffer: 1.0% BSA in PBS

Diluent: 1.0% BSA in PBS

RECOMMENDED MATERIALS:

ELISA microplates (Thermo Fisher Cat. # 456529);

BSA (Sigma Cat. # A-7030);

Stop Solution 2 M Sulfuric Acid (Sigma Cat. # 339741);

Dulbecco's PBS [10x] (Gibco BRL Cat. # 14200-075).

Storage

Store the kit at 4°C upon receipt. For more detailed information, please download the following document on our website.

Reconstitution And Storage

Capture Antibody: 360µg of antigen-affinity purified goat anti-M-CSF. Centrifuge vial prior to opening. Reconstitute in 1.0 ml PBS for a concentration of 360µg/ml. Following reconstitution the Capture antibodies may be stored at 2-8°Cfor up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Detection Antibody: 18 µg of biotinylated antigen-affinity purified goat anti-M-CSF. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile 1.0% BSA in PBS for a concentration of 18 µg/ml. Following reconstitution the Detection antibodies may be stored at 2-8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer.

Mouse M-CSF Standard: 25 ng of recombinant M-CSF. Centrifuge vial prior to opening. Reconstitute in 0.5 ml sterile 1.0% BSA in PBS for a concentration of 50 ng/ml. The Standard may be stored at 2-8°C for one month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer.

UltraAvidin-HRP Conjugate: 40µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2-8°C, DO NOT FREEZE.

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature and monitorcolor development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. The reaction may be stopped after 15-20 minutes by adding 100µl of 2 M sulfuric acid to each well.

Plate Preparation

1. Dilute to 2.0µg/ml of capture antibody and immediately add 100µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

Assay Procedure

Standard/Sample: Dilute standard from 2, 000 pg/ml to zero in diluent. Immediately add 100µl of standard or sample to each well in duplicate and incubate at room temperature for at least 2 hours.

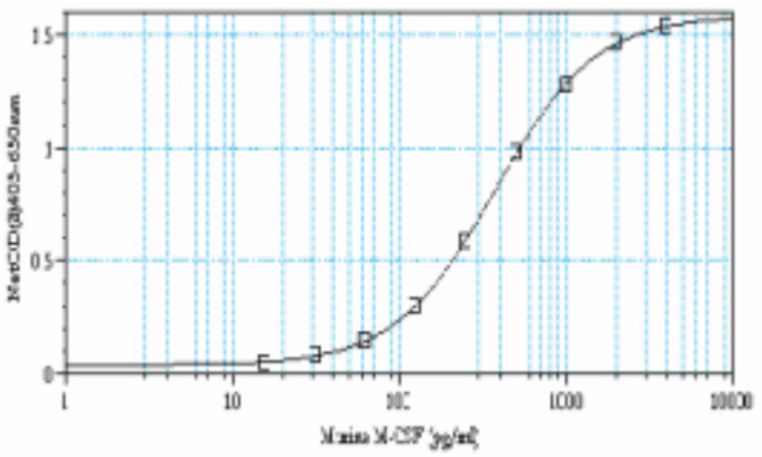
Detection: Aspirate and wash plate 4 times. Dilute a portion of detection antibody in diluent to a concentration of 50 ng/ml. Add 100µl per well and incubate at room temperature for 2 hours.

UltraAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 3.0 ul of avidin-HRP conjugate 1:5, 000 (this dilution factor may require some optimization) in diluent for a total volume of 15 ml. Add 100µl per well and incubate at room temperature for 30 minutes.

TMB Liquid Substrate: Add 100µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development with an ELISA plate reader at 450nm with wavelength correction set at 540nm or 570nm. Avoid placing plates in direct light.

Stop Solution: The reaction may be stopped after 15-20 minutes by adding 100µl of 2 M sulfuric acid (Sigma Cat. # 339741) to each well. All eye, hand, face, and clothing protection should be used when working with sulfuric acid.

Typical Standard Curve



The standard curve is provided as an example only. A standard curve should be prepared with each assay.

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