



User's Manual

Human GM-CSF ELISA Development Kit



CKERS-CSF2-023H



10 plates



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.

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PRODUCT INFORMATION

Intended Use

Human GM-CSF ELISA Development Kit contains the key components required for the quantitative measurement of natural and/or recombinant GM-CSF in a sandwich ELISA format. Using the ELISA protocol described below, this kit provides sufficient reagents to assay GM-CSF in approximately 1500 ELISA plate wells.

General Description

Human GM-CSF ELISA development kit is designed for the quantitative measurement within the range of 32-3000 pg/ml of natural or recombinant GM-CSF. Granulocyte-Macrophage Colony Stimulating Factor is a 22 kD, pleiotropic cytokine that is a white blood cell growth factor. It controls the production and function of blood cells by stimulating stem cells to produce granulocytes and monocytes. GM-CSF differs from G-CSF in that it affects more cell types including macrophages and eosinophils. Moreover, GM-CSF is part of the immune/inflammatory cascade, a process crucial for fighting infection. Interestingly, GM-CSF expression may have pathological implications. Autocrine expression of GM-CSF in myeloid leukemia cells is suspected to play a role in neoplasia, the formation of a new and abnormal growth of tissue. Additionally, GM-CSF expression has also been documented in certain solid tumors. There have also been reports of GM-CSF in synovial fluid from patients with arthritis suggesting that GM-CSF may play a role in tissue damage associated with the inflammatory process. Blocking GM-CSF is thought to have therapeutic potential by reducing inflammation. Some drugs are currently being developed to block GM-CSF. Applications include Sandwich ELISA. The term "sandwich" stems from the protein target being bound between two antibodies. The primary antibody is coated to the wells of the microplate. Samples, standards, or controls are then added into these wells, which bind to the immobilized primary antibody. The secondary antibody is added, binding to the primary antibody/target protein complex forming the "sandwich". Using the provided ELISA protocol, this kit provides sufficient reagents to assay GM-CSF in approximately 1500 ELISA plate wells.

Reagents And Materials Provided

1. Capture Antibody
2. Detection Antibody
3. Standard
4. StreptAvidin-HRP
5. TMB Liquid Substrate "Ready to Use"

Materials Required But Not Supplied

Additional Required Materials

ELISA microplates

BSA

Dulbecco's PBS (DPBS) [10×

Stop Solution: 450 nm Stop Reagent for TMB Microwell

Required Solutions

PBS: dilute 10×PBS to 1×PBS, pH 7.2, in sterile water

Wash Buffer: 0.05% Tween-20 in PBS

Reagent Diluent: 1.0% BSA in PBS*

Blocking Buffer 1.0% BSA in PBS

Note: Other acceptable blocking buffers such as CD's Ultra-FISH Block or Ultra-Sythetic Block may be used for assay optimization.

Storage

See "Reagent Preparation"

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS without carrier protein and immediately add 100 µL 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 300-400 µL of wash buffer per well.

Note: We recommend using an autowasher, although a squirt bottle or manifold dispenser would suffice.

3. After the last wash, invert plate to remove residual buffer and blot on paper towel.

4. Add 300 µL block buffer to each well and incubate for at least 1 hour at room temperature.

5. Aspirate and wash plate 4 times.

Note: Complete removal of liquid at each step is essential for good performance and sensitivity of assay.

Reagent Preparation

Mouse Anti-Human GM-CSF Capture Antibody: Centrifuge vial prior to opening. Reconstitute in 0.5 mL sterile PBS. Refer to the lot-specific datasheet for amount supplied and dilute in PBS without carrier protein to the working concentration indicated on the C of A. Following reconstitution the capture antibody 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. Avoid repeated freeze and thaw cycles.

Biotinylated Mouse Anti-Human GM-CSF Detection Antibody: Refer to the lot-specific datasheet for amount supplied. Centrifuge vial prior to opening. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A. 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 the freezer. Avoid repeated freeze and thaw cycles.

Recombinant Human GM-CSF Standard: Centrifuge vial prior to opening. Reconstitute each vial with 0.5 mL of deionized or distilled water. Refer to the lot-specific datasheet for amount supplied. The rProtein may be stored at 2-8°C for one (1) month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer. Avoid repeated freeze and thaw cycles.

TMB Liquid Substrate "Ready to Use" (TMB Substrate should be at ambient temperature prior to use): 60.0

mL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" is provided. The high quality of the substrate can be preserved by storing at temperatures between 2-8°C. When properly stored, TMB Microwell Substrate is stable for a minimum of 48 months from the manufactured date.

Assay Procedure

Standard/Sample: Add 100 µL of the working dilution with reagent dilution standard or sample to each well (duplicate recommended). Cover plate with an adhesive plate cover and incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Add 100 µL of the detection antibody, diluted in Reagent Diluent to each well. Cover with a new adhesive plate cover and incubate at room temperature for 2 hours.

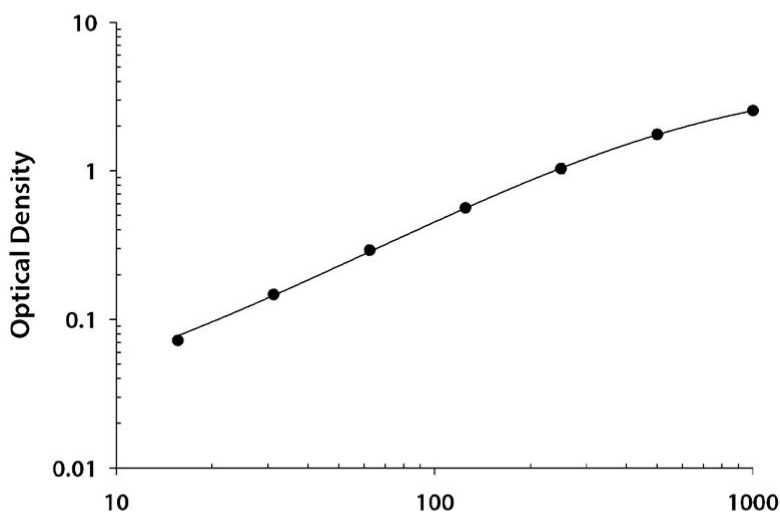
StreptAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Add 100 µL of the working dilution (the dilution factor may require optimization) to each well. Cover and incubate at room temperature for 20-30 minutes. Exposure to direct light should be avoided.

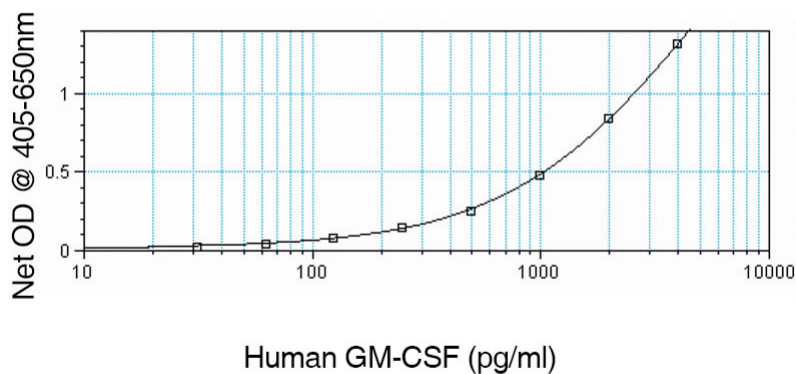
TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 µL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development. Exposure to direct light should be avoided.

Stop Solution: Add 50-100 µL of Stop Solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm.

Typical Standard Curve

Human GM-CSF Concentration (pg/mL)





Precautions

Some of the required components may contain acid and/or cause allergic reactions. Breathing in product mist or fumes should be avoided. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

References

1. Parker, MW. et al. (2008) Cell 134: 496
2. Whitsett, JA. et al. (2002) Annual Review of Physiology 64: 775