Human GM-CSF ELISA Kit

Introduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as CSF-2, is a pleiotropic cytokine with a molecular weight of 30 kDa and belongs to the βc cytokine family, which also includes IL-3 and IL-5. GM-CSF has anβhelix structure containing two intrachain disulfide bonds. A variety of activated immune cells, mesenchymal cells, and epidermal cells can secrete and produce GM-CSF. Mature human GM-SCF shares 54% and 63% amino acid homology with mouse GM-CSF and rat GM-CSF, respectively.

At present, this kit is mainly used to specifically detect the content of cytokines in human serum, plasma, cell or tissue lysate, or cell culture supernatant.

Compositions and storage

Components \ Size	96 Tests	Storage
Pre-coated plate (96-well, detachable)	1 plate	4°C
Positive Standard	7X 0.3 mL	-20°C
10X Washing Solution	50 mL	4°C
Enzyme-labeled anti-GM-CSF Reaction Solution	10 mL	4°C
TMB Solution	6 mL	4°C away from light
Stop Solution	16 mL	4°C
Sample Diluent Solution	6 mL	4°C
Shipping: Blue Ice	Shelf life: 6 months	

Protocol

1. Bring your own items for the experiment
   - Microplate reader (450 nm)
   - High precision sampler and gun head: 0.5-10 μL、2-20 μL、20-200 μL、200-1000 μL
   - 37°C incubator
   - Distilled water or deionized
2. Preparation of assay samples

2.1 Serum: Whole blood samples were centrifuged at 5,000 rpm for 5 minutes at room temperature for 2 h or 4°C overnight, and the supernatant was taken for detection.

2.2 Plasma: Anticoagulant recommends the use of EDTA sodium salt, centrifugation at 5,000 rpm for 5 min within 30 min after sample collection, and the supernatant can be taken for detection. Avoid using hemolytic, hyperlipidemic samples.

2.3 Tissue homogenate: Rinse the tissue with pre-chilled PBS (0.01 M, pH = 7.4) to remove residual blood and mince the tissue after weighing. The minced tissue is compared to the corresponding volume of PBS (generally a weight-to-volume ratio of 1:9, for example, 1 g of tissue sample corresponds to 9 mL of PBS, and the specific volume can be adjusted appropriately according to the needs of the experiment, and a good record should be made. It is recommended to add protease inhibitors to PBS) to a glass homogenizer and grind well on ice. To further lyse the histiocytes, the homogenate can be sonicated or repeatedly freeze-thawed. Finally, the homogenate was centrifuged at 5,000 rpm for 5-10 minutes, and the supernatant was taken for testing.

2.4 Cell extraction: Adherent cells were gently washed with cold PBS, then trypsinized, and cells were collected after centrifugation at 1,000 rpm for 5 minutes; Suspension cells can be collected by direct centrifugation. Collected cells are washed 3 times with cold PBS. Add 150-200 μL of PBS to every 106 cells per 1× to resuspend and disrupt the cells (reduce the volume of PBS if the content is very low). The extract was centrifuged at 2,000 rpm for 10 minutes, and the supernatant was taken for testing.

2.5 Cell culture supernatant or other biological fluids: Centrifuge at 2,000 rpm for 20 minutes to remove impurities and cell debris. Take the supernatant for testing.

***Note:** NaN_3 can affect and inhibit horseradish peroxidase (HRP) activity, so samples containing NaN_3 cannot be detected.

3. Experimental Procedure

3.1 The number of Pre-coated plates (96-well, detachable) required for an experiment is calculated and determined. The required plates are taken out and placed in the 96-well frame. The remaining microcoated plates are put back in the aluminum foil bag and sealed and stored at 4℃.

3.2 Set standard holes, sample holes and blank holes; Add Positive Standard 100 μL with different concentration in each standard hole; Add 100 μL of the sample to be measured into the sample hole; Add Sample Diluent Solution 100μL in blank holes.

***Note:** The concentrations of the standards were 500, 250, 125, 62.5, 31.25, 15.625, and 7.813 ng/mL from high to low.

3.3 After incubation at 37℃ for 1 h, 10× Washing Solution was diluted with ddH2O to 1× 200μL/well, washed 3 times, and patted dry each time.

3.4 Add Enzyme-labeled anti-GM-CSF Reaction Solution, 100 μL/ well, and incubate at room temperature for 1 h.

3.5 1 x Washing Solution, same as 3.3.

3.6 Add TMB Solution 50 μL/well and incubate at room temperature for 5 min away from light.

3.7 Add Stop Solution 150 μL/well, read at a wavelength of 450 nm, and record the results. The assay should be performed within 15 minutes of the addition of the stop solution.

4. Analysis of results

4.1 Values for double wells are usually valid within a 15% difference range, and the average value of the double wells can be used as a measured value.

4.2 The absorbance value of each standard or sample should be subtracted from the absorbance value of the background correction well (if no correction hole is made, it is not required).

4.3 Draw a standard curve. The concentration of the standard is used as the abscissa, and the A450 value is used as the ordinate, and the coordinate points of each standard are connected by a smooth line. The corresponding concentration of the sample is calculated from the absorbance value of the sample and the standard curve (straight fit).

4.4 If the OD value of the sample is higher than the upper limit of the standard curve, it should be diluted appropriately and re-measured, and the concentration should be multiplied by the dilution factor of the sample.

Note

1. The operation is strictly in accordance with the instructions, and the kits with different batch numbers of this product cannot be mixed.
2. When dispensing, please note that tips must be changed for each sample or standard to avoid cross-contamination and aspiration volume errors.
3. All reagents in each group were stored at 4°C, equilibrated to room temperature before use, and unpacked unused plates can be vacuumed and stored at 4°C or frozen at -20°C.
4. The chromogenic solution of this substrate should be stored in the dark and should not be exposed to strong light.
5. The stop solution of this product is 1 M sulfuric acid, and it must be used safely, so as not to cause the liquid to splash on the bare skin.
6. This product is for scientific research use only.

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