Fast Mouse GM-CSF ELISA

For the quantitative determination of mouse GM-CSF concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

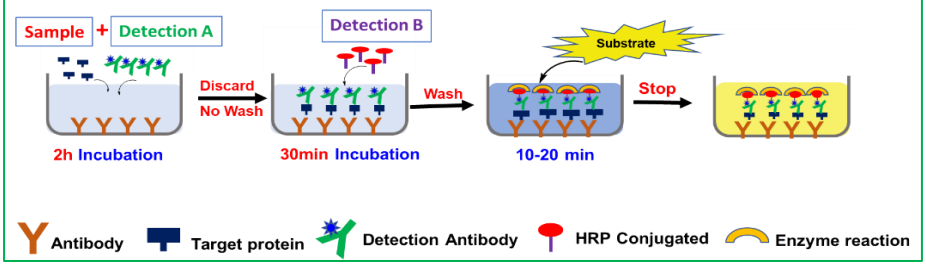
Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), also known as CSF-2, is a pleiotrophic 30 kDa member of the Common beta Chain (βc) cytokine family that also includes IL-3 and IL-5. It is secreted by a wide variety of activated immune, mesenchymal, and epithelial cell types and circulates as a variably glycosylated monomer. It is upregulated in multiple cell types during inflammation including encephalitogenic T cells, allergen exposed lung endothelial cells, and IgE activated mast cells. It induces monocyte, neutrophil, and eosinophil production from CD34+ stem cell precursors. GM-CSF promotes Th1 and Th17 cell mediated autoimmune inflammation as well as the inflammatory activation of dendritic cells, microglia, alveolar macrophages, and eosinophils. In addition, it cooperates with G-CSF in promoting tumor cell proliferation and invasion.

The Mouse GM-CSF ELISA is designed to quantitatively detect Mouse GM-CSF levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 2 hours, not need 4-5 hours (Fig. 1). **The detection range is from 7.8 to 500 pg/mL**. The levels of GM-CSF samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural GM-CSF protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for mouse GM-CSF was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then, incubated with HRP-conjugated detection antibody specific for mouse GM-CSF. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is in proportion to the amount of GM-CSF bound in the initial step. The intensity of the color is measured by plate read at 450.

Fig. 1 Simple Assay Procedures



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse GM-CSF Capture	TBS3079A	96 well microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse GM-CSF	Aliquot and store at 4-8℃ for 3 months.
Mouse GM-CSF Standard	TBS3079B	10µl of Recombinant mouse GM-CSF protein (80ng/mL).	Aliquot and store at -20 ℃ for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3079C	2.1 mL of Biotin-mouse GM-CSF antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3079D	200 µL of Streptavidin-HRP (50x).	
Assay Diluent	TBS3000E	20 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on 3x 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).*

Detection B: Dilute 200 µL Detection B stock with Assay Diluent to 10mL as a working solution of Detection B.

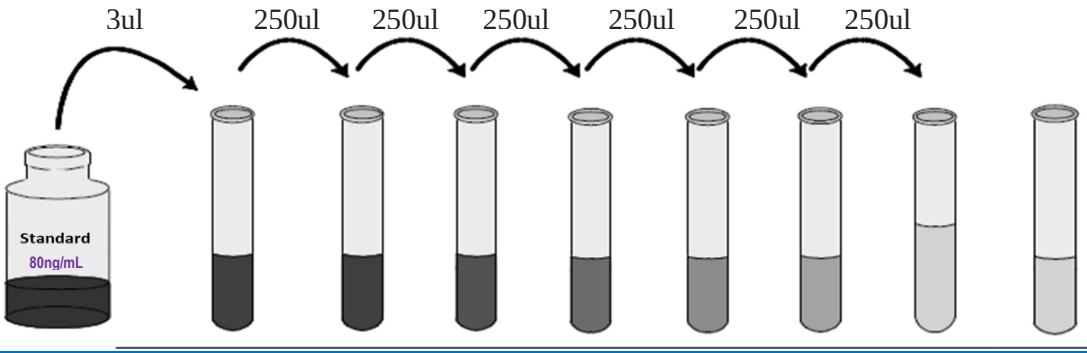
Mouse GM-CSF Standard Preparation:

Label test tubes as #1 through #8. Pipet 477 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as diagram below.**

1. Add 3 µL of the Mouse GM-CSF Standard stock solution (80ng/mL) to tube #1 (500 pg/mL), and mix.

2. Make 2x serial dilutions of the standard using the 500pg/mL standard solution from tube #2 through #7 with sequential transfer of 500 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 500, 250, 125, 62.5, 31.25,15.625, and 7.81pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Mouse GM-CSF standard preparation



Standard Label	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	477	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	3	250	250	250	250	250	250	0
Final Conc (pg/ml)	500	250	125	62.5	31.25	15.625	7.81	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hour**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 30min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at

540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse GM-CSF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve(R^2=0.9998) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of Mouse GM-CSF is typically 7pg/ml.

The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

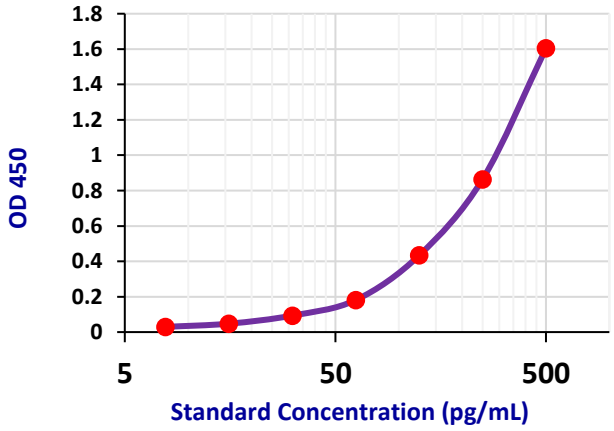
SPECIFICITY

This assay recognizes natural and recombinant mouse GM-CSF. No cross-reactivity with other cytokines.

RELATIVE PRODUCTS

TBS3030	Fast Mouse IL-1β ELISA
TBS3031	Fast Mouse IL-4 ELISA
TBS3032	Fast Mouse IL-4 ELISA
TBS3040	Fast Mouse IL-6 ELISA
TBS3044	Fast Mouse IL-10 ELISA
TBS3047	Fast Mouse IL-12 p70 ELISA
TBS3049	Fast Mouse IL-13 ELISA
TBS3060	Fast Mouse KC ELISA
TBS3070	Fast Mouse NGF ELISA
TBS3080	Fast Mouse G-CSF ELISA
TBS3084	Fast Mouse IFN-γ ELISA
TBS3085	Fast Mouse TGF ELISA
TBS3086	Fast Mouse MCPT-1 ELISA
TBS3090	Fast Mouse IL-17AF ELISA
TBS3091	Fast Mouse IL-19 ELISA
TBS3092	Fast Mouse IL-21 ELISA
TBS3093	Fast Mouse IL-22 ELISA
TBS3094	Fast Mouse IL-23 ELISA
TBS3095	Fast Mouse IL-27 ELISA
TBS3096	Fast Mouse IL-28B ELISA
TBS3097	Fast Mouse IL-33 ELISA
TBS3098	Fast Mouse Insulin ELISA

Fig3. Mouse GM-CSF Standard



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