

Mouse GM-CSF ELISA Kit

EMGMCSF EMGMCSF2

1340.4

Number	Description
EMGMCSF	<p>Mouse Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) ELISA Kit, sufficient reagents for 96 determinations</p> <p>Kit Contents:</p> <p>Anti-Mouse GM-CSF Precoated 96-well Strip Plate, 1 each</p> <p>Lyophilized Recombinant Mouse GM-CSF Standard, 2 vials</p> <p>Standard Diluent, 1 vial</p> <p>Plate Reagent, 1 vial</p> <p>30X Wash Buffer, 1 vial</p> <p>Prediluted Conjugate Reagent, 1 vial</p> <p>TMB Substrate, 1 vial</p> <p>Stop Solution, 13 ml, 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 6 each</p>
EMGMCSF2	<p>Mouse GM-CSF ELISA Kit, sufficient reagents for 2 × 96 determinations</p> <p>Kit Contents:</p> <p>Anti-Mouse GM-CSF Precoated 96-well Strip Plate, 2 each</p> <p>Lyophilized Recombinant Mouse GM-CSF Standard, 4 vials</p> <p>Standard Diluent, 2 vial</p> <p>Plate Reagent, 2 vial</p> <p>30X Wash Buffer, 2 vial</p> <p>Prediluted Conjugate Reagent, 2 vial</p> <p>TMB Substrate, 2 vial</p> <p>Stop Solution, 2 × 13 ml, 0.16 M sulfuric acid</p> <p>Adhesive Plate Covers, 12 each</p>

For research use only – not for use in diagnostic procedures.

Storage: For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.




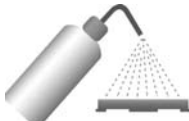

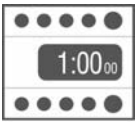






Introduction

This Thermo Scientific ELISA Kit is for the quantitative measurement of mouse GM-CSF in serum and culture supernatants.

Table of Contents

Introduction	1
Procedure Summary.....	2
Additional Materials Required.....	2
Precautions.....	3
Sample Preparation.....	3
Reagent Preparation.....	4
Assay Procedure	4
Performance Characteristics	6
Reference	6
Data Templates	7

Procedure Summary

- | | | | |
|---|---|--|---|
|  |  |  |  |
| 1. Add 50 μ l of Plate Reagent to each well. | 2. Add 50 μ l of diluted Standards or Samples. | 3. Cover plate and incubate for 2 hours at 37°C in a humidified incubator. | 4. Wash the plate FIVE times, then pat dry. |
|  |  |  |  |
| 5. Add 100 μ l of Prediluted Conjugate Reagent to each well. | 6. Cover plate and incubate at 37°C for 1 hour in humidified incubator. | 7. Wash the plate FIVE times, then pat dry. | 8. Add 100 μ l TMB Substrate to each well. |
|  |  |  |  |
| 9. Develop the plate in the dark at room temperature (20-25°C) for 30 minutes. | 10. Stop reaction by adding 100 μ l of the Stop Solution to each well. | 11. Measure absorbance on a plate reader at 450 nm or 450 minus 550 nm. | 12. Calculate results using curve-fitting statistical software. |

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 0.05 to 1.00 ml (+/- 1%)
- Plastic pipettes to deliver 5 to 15 ml (+/- 1%)
- Ultrapure water for Wash Buffer and Standard reconstitution
- A glass or plastic 2 liter container to prepare Wash Buffer
- A squirt wash bottle or an automated immunoplate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- 15 ml plastic tube to prepare Conjugate
- 5 disposable reagent reservoirs
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance can be read at 450 nm only. Refer to the instruction manual supplied with your instrument.
- Graph paper or a curve-fitting statistical software package

Precautions

- All specimens and reagents must be at room temperature (20-25°C) before use in the assay
- Carefully review all instructions and verify components against the Kit Contents list (page 1) before beginning the assay.
- Vigorous washing of plate after the Sample and Conjugate incubation steps is essential.
- Do not use water a bath to thaw samples or reagents. Thaw samples and reagents at room temperature.
- If using a multichannel pipettor, always use a new disposable reagent reservoir when adding Plate Reagent, Conjugate Reagent, TMB Substrate Solution and Stop Solution.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- For sample and conjugate incubations use a humidified 37°C incubator.
- Do not mix reagents from different kit lots. Discard unused ELISA components after assay completion.
- Avoid microbial contamination of reagents.
- Avoid exposure of reagents to excessive heat or light during storage and incubation.
- If using samples that are clotted, grossly hemolyzed or microbially contaminated, or if there is any question about the integrity of a sample, make a note on the template and interpret results with caution.
- Individual components may contain preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents.
- Do not use glass pipettes to measure the TMB Substrate Solution.
- Be careful not to contaminate the TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT.

Sample Preparation

Sample Handling

- Serum or culture supernatants may be tested in this ELISA.
- Use 50 µl of sample per well.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Assay test samples in duplicate each time the ELISA is performed.
- Avoid freezing and thawing samples more than once.
- Gently equilibrate samples to room temperature before performing assay. Mix samples by gently inverting tubes. Do not use a heated water bath to thaw samples.

Sample Dilution

- If the GM-CSF concentration of a sample possibly exceeds the highest point of the standard curve (i.e., 250 pg/ml), prepare one or more five-fold dilutions of the test sample. For example, prepare a five-fold dilution by adding 100 µl of test sample to 400 µl of appropriate diluent.
- When testing **culture supernatants**, prepare serial dilutions using the culture medium.
- When testing **serum**, prepare serial dilutions using the Standard Diluent provided.
- Mix thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using a partial plate, look for **(PP)** throughout these instructions.

Wash Buffer

1. Label a clean glass or plastic 2 liter container “Wash Buffer.”
2. Add the entire contents of the 30X Wash Buffer bottle and dilute with ultrapure water to a final volume of 1.5 liters. Mix thoroughly. **Wash Buffer must be at room temperature before use in the assay.**

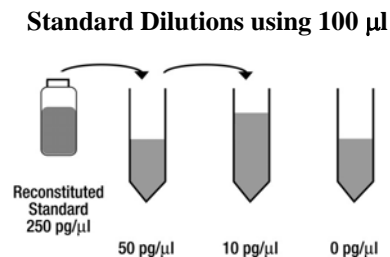
(PP) When using a partial plate, store the reconstituted Wash Buffer at 2-8°C. **Do not use Wash Buffer if it becomes visibly contaminated during storage.**

Standards

- **(PP)** Reconstitute and use one vial per partial plate.
 - Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
1. When testing culture supernatant samples, reconstitute the Standard with sample culture medium to the volume stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting the vial. The concentration will be 250 pg/ml, which is the highest point of the standard curve. Use the sample culture medium to prepare Standard Curve dilutions.

When testing serum samples, reconstitute standard with ultrapure water to the volume stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting the vial. The concentration will be 250 pg/ml, which is the highest point of the standard curve. Use the Standard Diluent provided to prepare serial dilutions of the Standard Curve.

2. Label 3 tubes, one for each of the standard dilutions: 50 pg/ml, 10 pg/ml and 0 pg/ml.
3. Pipette 400 µl of appropriate diluent (as described above) into each tube.
4. Pipette 100 µl of reconstituted Standard into the first (i.e., 50 pg/ml) tube and mix.
5. Pipette 100 µl of this dilution into the second (i.e., 10 pg/ml) tube and mix.



Assay Procedure

A. Sample Incubation

Notes:

- Use the Data Template provided to record locations of the zero standard (blank or negative control), mGM-CSF Standards and test samples. Perform three Standards and one blank in duplicate with each series of unknown samples.
- If using a multichannel pipettor, use a new reagent reservoir to add the Plate Reagent.
- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Place remaining unused strips in the provided foil pouch with desiccant. Tightly seal the foil pouch and store at 2-8°C. After assay completion, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.

1. Add 50 µl of Plate Reagent to each well.
2. Add 50 µl of diluted standards or test samples in duplicate to each well.

Note: If the mGM-CSF concentration in any test sample possibly exceeds the highest point on the standard curve 250 pg/ml, see Sample Preparation – Sample Dilution section.

3. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are sealed tightly by running your thumb over edges and down each strip. Incubate for two (2) hours at 37°C in a humidified incubator.
4. Carefully remove adhesive plate cover. Wash plate FIVE times with Wash Buffer as described in the Plate Washing section (Section B).

B. Plate Washing

1. Gently squeeze the long sides of the plate frame before washing to ensure all strips securely remain in the frame.
2. Discard plate contents. Use a squirt wash bottle to vigorously fill each well completely with Wash Buffer, then discard plate contents. Repeat procedure four additional times for a total of FIVE washes. Blot plate onto paper towels or other absorbent material.

Note: Automated plate washers may produce sub-optimal results. For best results, perform a manual wash the first time the assay is performed. Automated plate washing may require validation to determine the number of washes necessary to achieve optimal results; typically, five washes are sufficient. For automated washing, aspirate all wells and wash by overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Conjugate Incubation

1. Use a single or multichannel pipettor to add 100 μ l of Prediluted Conjugate Reagent to each well. If using a multichannel pipettor, use a new reagent reservoir when adding Conjugate Reagent.
2. Carefully attach a new adhesive plate cover. Make sure all edges and strips are sealed tightly. Incubate plate for one (1) hour at 37°C in a humidified incubator.
3. Carefully remove adhesive plate cover and wash **FIVE** times as described in the Plate Washing section.

D. Substrate Incubation and Stop Step

- Use **new disposable reagent reservoirs** when adding the TMB Substrate and Stop Solution.
 - Dispense from bottle **ONLY** the amount required, 100 μ l per well, for the number of wells being used. **Do not use a glass pipette** to measure the TMB Substrate.
 - **(PP) Do not combine leftover substrate** with that reserved for the second partial plate. Take care not to contaminate remaining TMB Substrate Solution.
1. Pipette 100 μ l of TMB Substrate Solution into each well.
 2. Allow enzymatic color reaction to develop at room temperature for 30 minutes. Develop plate in the dark. Do not cover plate. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
 3. After 30 minutes, stop the reaction by adding 100 μ l of Stop Solution to each well.

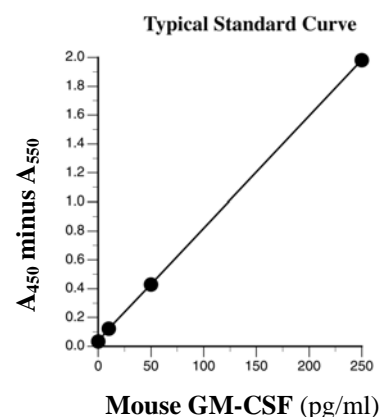
E. Absorbance Measurement

The plate must be evaluated within 30 minutes of stopping the reaction. Measure absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from at 450 nm values to correct for optical imperfections in the microplate. If 550 nm is not available, measure absorbance at 450 nm only.

Note: Omitting the 550 nm measurement will in higher absorbance values.

F. Results Calculation

- Use the standard curve to determine mGM-CSF amount in an unknown sample. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding mGM-CSF concentration (pg/ml) on the horizontal (X) axis.
- Calculate results manually using graph paper or curve-fitting statistical software. Determined the mGM-CSF amount in each sample by interpolating from the absorbance value (Y axis) to mGM-CSF concentration (X axis).
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate pg/ml of mGM-CSF in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.



Performance Characteristics

Sensitivity:

<5 pg/ml of mouse GM-CSF

Specificity:

Natural and recombinant mouse GM-CSF. This ELISA does not cross-react with mouse IL-2, IL-3, IL-4, IL-5, IL-6, IFN γ , TNF α , rabbit GM-CSF, rat GM-CSF or human GM-CSF.

Assay Parameters

Assay Range:

0-250 pg/ml

Suggested curve points are 0, 10, 50 and 250 pg/ml

Reproducibility:

Intra-assay CV: <10%

Inter-assay CV: <10%

Calibration:

The standards in this ELISA are calibrated to NIBSC reference lot 91/658.

One (1) Endogen pg = 1.52 NIBSC pg.

Reference

In *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds., Academic Press: New York, p71.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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Data Templates

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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