INSTRUCTIONS

Mouse GM-CSF ELISA Kit

EMGMCSF EMGMCSF2 EMGMCSF5

1340.6

Number	Description
EMGMCSF	**Mouse Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) ELISA Kit,** sufficient reagents for 96 determinations
EMGMCSF2	**Mouse GM-CSF ELISA Kit,** sufficient reagents for 2 × 96 determinations
EMGMCSF5	**Mouse GM-CSF ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EMGMCSF	EMGMCSF2	EMGMCSF5
Anti-mouse GM-CSF Pre-coated 96-well Strip	1 each	2 each	5 each
Lyophilized Recombinant Mouse GM-CSF	2 vials	4 vials	10 vials
Standard Diluent	12mL	2 × 12mL	5 × 12mL
Plate Reagent	8mL	2 × 8mL	5 × 8mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Prediluted Conjugate Reagent	12mL	2 × 12mL	5 × 12mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate sealers	6 each	12 each	30 each

For research use only. Not for use in diagnostic procedures.

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

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Introduction

The Thermo Scientific™ Mouse GM-CSF ELISA Kit measures mouse GM-CSF in serum and culture supernatants.

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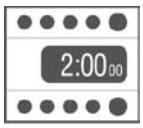
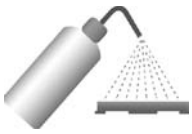
Procedure Summary

1. Add 50µL of Plate Reagent to each well.

2. Add 50µL 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 100µL of Prediluted Conjugate Reagent to each well.

6. Cover plate and incubate at 37°C for 1 hour in humidified incubator.

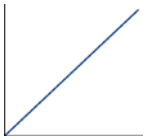
7. Wash the plate FIVE times, then pat dry.

8. Add 100µL 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 100µL of the Stop Solution to each well.

11. Measure absorbance on a plate reader at 450nm or 450 minus 550nm.



12. Calculate results using curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 0.05 to l.00mL (+/- 1%)
- Plastic pipettes to deliver 5 to 15mL (+/- 1%)
- Ultrapure water for Wash Buffer and Standard reconstitution
- A glass or plastic 2L container to prepare Wash Buffer
- A squirt wash bottle or an automated immunoplate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- 15mL plastic tube to prepare Conjugate
- Five disposable reagent reservoirs
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be read at 450nm 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.

Additional Precaution for the 2-plate and 5-plate Kits

- Dispense, pool, and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.

Sample Preparation

Sample Handling

- Serum or culture supernatants may be tested in this ELISA.
- Use 50µL 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., 250pg/mL), prepare one or more five-fold dilutions of the test sample. For example, prepare a five-fold dilution by adding 100µL of test sample to 400µL 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 2L container "Wash Buffer."
2. Add the entire contents of the 30X Wash Buffer bottle and dilute with ultrapure water to a final volume of 1.5L. 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.**

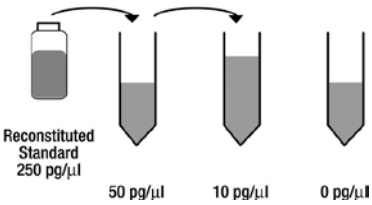
Standards

- **(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 250pg/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 250pg/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: 50pg/mL, 10pg/mL and 0pg/mL.
3. Pipette 400µL of appropriate diluent (as described above) into each tube.
4. Pipette 100µL of reconstituted Standard into the first (i.e., 50pg/mL) tube and mix.
5. Pipette 100µL of this dilution into the second (i.e., 10pg/mL) tube and mix.

Standard Dilutions using 100µL



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 50µL of Plate Reagent to each well.
2. Add 50µL 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 250pg/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 100µL 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, 100µL 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 100µL 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 100µL of Stop Solution to each well.

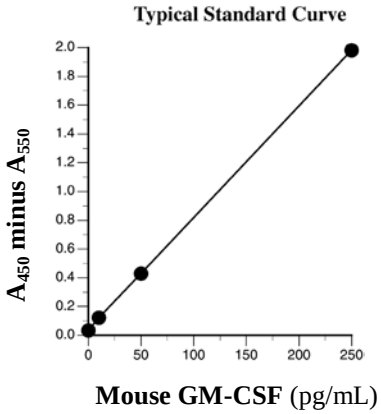
E. Absorbance Measurement

The plate must be evaluated within 30 minutes of stopping the reaction. Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550 nm values from at 450nm values to correct for optical imperfections in the microplate. If 550nm is not available, measure absorbance at 450nm 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:
< 5pg/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, IFNγ, TNFα, rabbit GM-CSF, rat GM-CSF or human GM-CSF.

Assay Parameters

Assay Range:
0-250pg/mL
Suggested curve points are 0, 10, 50 and 250pg/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.

Cited Reference

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

Data Templates

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