INSTRUCTIONS

Human GM-CSF ELISA Kit

EHGMCSF EHGMCSF2 EHGMCSF5

1359.4

Number	Description
EHGMCSF	**Human Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) ELISA Kit,** sufficient reagents for 96 determinations
EHGMCSF2	**Human GM-CSF ELISA Kit,** sufficient reagents for 2 × 96 determinations
EHGMCSF5	**Human GM-CSF ELISA Kit,** sufficient reagents for 5 × 96 determinations

Kit Contents	EHGMCSF	EHGMCSF2	EHGMCSF5
Anti-human GM-CSF Precoated 96-well Strip Plate	1 each	2 each	5 each
Lyophilized Recombinant Human GM-CSF	2 vials	4 vials	10 vials
Standard Diluent	12mL	2 × 12mL	5 × 12mL
Biotinylated Antibody Reagent	8mL	2 × 8mL	5 × 8mL
30X Wash Buffer	50mL	2 × 50mL	5 × 50mL
Streptavidin-HRP Concentrate	75µL	2 × 75µL	5 × 75µL
Streptavidin-HRP Dilution Buffer	14mL	2 × 14mL	5 × 14mL
TMB Substrate	13mL	2 × 13mL	5 × 13mL
Stop Solution, contains 0.16M sulfuric acid	13mL	2 × 13mL	5 × 13mL
Adhesive plate covers	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™ Human GM-CSF ELISA Kit measures human GM-CSF in serum, plasma, urine and culture supernatants.

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Procedure Summary

1. Add 50µL Biotinylated Antibody Reagent to each well.

2. Add 50µL of Standards or samples to each well in duplicate.

3. Cover plate and incubate at room temperature (20-25°C) for 3 hours.

4. Wash plate THREE times.

5. Add 100µL of prepared Streptavidin-HRP Solution to each well.

6. Cover plate and incubate at room temperature for 30 minutes.

7. Wash plate THREE times.

8. Add 100µL of Premixed TMB Substrate Solution to each well.

9. Develop plate in the dark at room temperature for 30 minutes.

10. Stop reaction by adding 100µL of Stop Solution to each well.

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

12. Calculate results using graph paper or curve-fitting statistical software.

Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL
- Plastic pipettes to deliver 5-15mL
- Ultrapure water
- A glass or plastic 2L container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- 15mL plastic tube to prepare Streptavidin-HRP Solution
- Microcentrifuge to prepare Streptavidin-HRP Solution
- A standard ELISA reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance can be measured at 450nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

Precautions

- **All samples and reagents must be at room temperature (20-25°C) before use in the ELISA.**
- Review these instructions carefully and verify all components against the Kit Contents list (page 1) before beginning the assay.
- Do not use water baths to thaw samples. Thaw at room temperature.
- When preparing standard curve and sample dilution in culture medium, use the same medium used to culture the cells. For example, if RPMI with 10% fetal calf serum (FCS) was used to culture cells, then use RPMI with 10% FCS to dilute the standard and samples. Do NOT use RPMI without serum supplement.

- If using a multichannel pipettor, always use a new disposable reagent reservoir.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Once reagents have been added to the plate, take care NOT to let plate DRY at any time during the assay.
- Avoid microbial contamination of reagents.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Discard unused kit components. Do not mix reagents from different kit lots.
- Do not use glass pipettes to measure TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.

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; EDTA, sodium citrate and heparin plasma; urine; or culture supernatants may be tested in this ELISA.
- 50µL per well of serum, plasma, urine or culture supernatant are required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C.
- Avoid repeated freeze-thaw cycles when storing samples.
- Test samples and standards must be assayed in duplicate each time the ELISA is performed.
- Gradually equilibrate samples to room temperature before beginning assay. Do not use heated water baths to thaw or warm samples.
- Mix samples by gently inverting tubes.
- If samples are clotted, grossly hemolyzed, lipemic 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.

Sample Dilution

- If the human GM-CSF concentration possibly exceeds the highest point of the standard curve (i.e., 600pg/mL), prepare one or more 10-fold dilutions of the test sample. When testing **culture supernatants**, prepare the serial dilutions using the culture medium. When testing **serum, plasma or urine,** prepare the serial dilutions using the Standard Diluent provided. For example, a 10-fold dilution is prepared by adding 0.05mL (50µL) of test sample to 0.45mL (450µL) of appropriate diluent. Mix thoroughly between dilutions before assaying.

Reagent Preparation

For procedural differences when using partial plates, look for **(PP)** throughout this instruction booklet.

Wash Buffer

- Label a clean glass or plastic two-liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
- Add the entire contents of the 30X Wash Buffer (50mL) bottle to the 2L container and dilute to a final volume of 1.5L with ultrapure water. Mix thoroughly.

  (PP) When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

  Note: Wash Buffer must be at room temperature before use in the assay. Do not use Wash Buffer if it becomes visibly contaminated during storage.

Standards

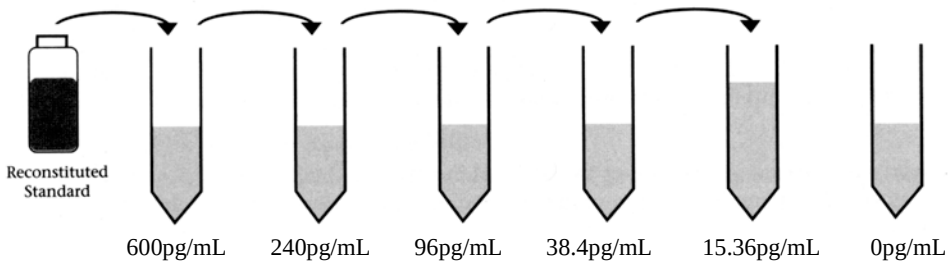
- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
- Prepare Standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
- When testing **culture supernatant samples,** reconstitute standard with ultrapure water. Reconstitution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

  When testing **serum, plasma or urine samples,** reconstitute standard with ultrapure water. Reconstitution volume is stated on the standard vial label. The standard will dissolve in approximately 1 minute. Mix by gently inverting vial. Use the Standard Diluent provided to prepare standard curve serial dilutions.

  When testing **serum, plasma or urine and cell culture supernatant samples on the same plate,** validate the media to establish if the same standard curve can be used for the different sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Standard Diluent provided. If the OD values of the two curves are within 10% of the mean for both curves, the assay can be performed with Standard Diluent, whether you are testing culture supernatant, urine, plasma, or serum samples.

1. Label six tubes, one for each standard curve point: 600pg/mL, 240pg/mL, 96pg/mL, 38.4pg/mL, 15.36pg/mL and 0pg/mL, then prepare 1:2.5 serial dilutions for the standard curve as follows:
2. Pipette 240µL of appropriate diluent into each tube.
3. Pipette 160µL of the reconstituted standard into the first tube (i.e., 600pg/mL) and mix.
4. Pipette 160µL of this dilution into the second tube (i.e., 240pg/mL) and mix.
5. Repeat the serial dilutions (using 160µL) three more times to complete the standard curve points.

Serial dilutions using 160µL



Assay Procedure

A. Biotinylated Antibody Reagent and Sample Incubation

- **(PP)** Determine number of strips required. Leave these strips in the plate frame. Tightly seal remaining unused strips in the provided foil pouch with desiccant and store at 2-8°C. After completing assay, retain plate frame for second partial plate. When using the second partial plate, place strips securely in the plate frame.
- Use the Data Template provided to record the locations of the zero standard (blank or negative control), human GM-CSF standards and test samples. Perform five standard points and one blank in duplicate with each series of unknown samples.
- If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent. Remove from the vial only the amount required for the number of strips being used. Take care not to touch the samples in wells with the pipette tip when adding the Biotinylated Antibody Reagent.

1. Add 50µL of Biotinylated Antibody Reagent to each well.
2. Add 50µL of reconstituted standards or test samples in duplicate to each well. Mix well by gently tapping the plate several times.

   Note: If the human GM-CSF concentration in any test sample exceeds the highest point on the standard curve, 600pg/mL, see Sample Preparation-Sample Dilution section.
3. Add 50µL of Standard Diluent to all wells that do not contain standards or samples.
4. 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 three (3) hours at room temperature, 20-25°C.
5. Carefully remove adhesive plate cover. Wash plate THREE times with Wash Buffer as described in the Plate Washing section (section B).

B. Plate Washing

- Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
- **Manual Wash:** Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.
- **Automated Wash:** Aspirate all wells and wash THREE times with Wash Buffer, overfilling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

C. Streptavidin-HRP Solution Preparation and Incubation

- Prepare Streptavidin-HRP Solution **immediately before use**. Do not prepare more Streptavidin-HRP Solution than required.
- Do not store prepared Streptavidin-HRP Solution.
- Use a 15mL plastic tube to prepare Streptavidin-HRP Solution.
- **Note:** If using a multichannel pipettor, **use new reagent reservoir and pipette tips** when adding the prepared Streptavidin-HRP Solution.

1. Briefly centrifuge the Streptavidin-HRP Concentrate to force entire vial contents to the bottom.
2. **(PP)** Use only the Streptavidin-HRP Solution amount required for the number of strips being used. For each strip, mix 2.5µL of Streptavidin-HRP Concentrate with 1mL of Streptavidin-HRP Dilution Buffer. Store Streptavidin-HRP Concentrate reserved for additional strips at 2-8°C.

   For one complete 96-well plate, add 30µL of Streptavidin-HRP Concentrate to 12mL Streptavidin-HRP Dilution Buffer and mix gently.
3. Add 100µL of prepared Streptavidin-HRP Solution to each well.

4. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
5. Carefully remove the adhesive plate cover, discard plate contents and wash THREE times as described in the Plate Washing Section (section B).

D. Substrate Incubation and Stop Step

- Use new disposable reagent reservoirs when adding TMB Substrate Solution and Stop Solution.
- Dispense from bottle ONLY amount required, 100µL per well, for the number of wells being used. Do not use a glass pipette to measure the TMB Substrate Solution.
- **(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 the color reaction to develop at room temperature in the dark for 30 minutes. Do not cover plate with aluminum foil or a plate sealer. 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

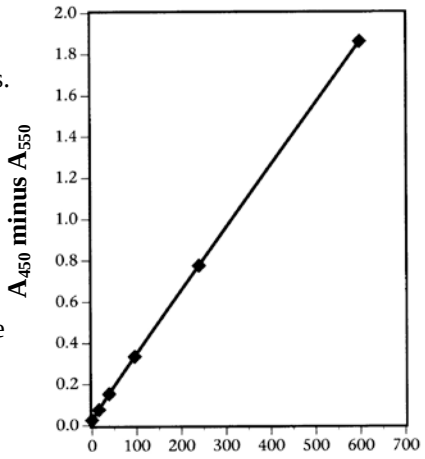
Note: Evaluate the plate within 30 minutes of stopping the reaction.

Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If an absorbance at 550nm is not available, measure at 450nm only. When the 550nm measurement is omitted, absorbance values will be higher.

F. Calculation of Results

- The standard curve is used to determine human GM-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 human GM-CSF concentration (pg/ml) on the horizontal (X) axis.
- Calculate results using graph paper or curve-fitting statistical software. Determine the human GM-CSF amount in each sample by interpolating from the absorbance value (Y axis) to human GM-CSF concentration (X axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value obtained from the standard curve by the dilution factor to calculate pg/ml of human GM-CSF in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Duplicate values that differ from the mean by greater than 10% should be considered suspect and repeated.

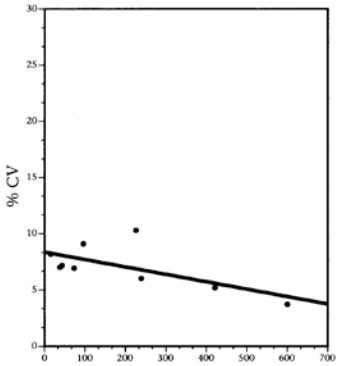
Standard Curve Example



Performance Characteristics

Sensitivity: < 2pg/mL
The sensitivity or Lower Limit of Detection (LLD)[1] is determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

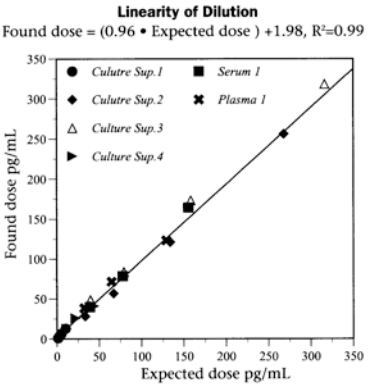
Assay Range: 15.36-600pg/mL
Suggested standard curve points are 0, 15.36, 38.4, 96, 240, and 600pg/mL.
Precision: The intra-assay coefficient of variation is plotted against GM-CSF concentration (pg/mL). The points represent samples evaluated in replicates of four in four different kit lots.



Reproducibility:
Intra-assay CV: < 10%
Inter-assay CV: < 10%

Specificity: This ELISA is specific for the measurement of natural and recombinant human GM-CSF. It does not cross-react with human IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, IFNγ, TNFα, or mouse GM-CSF.

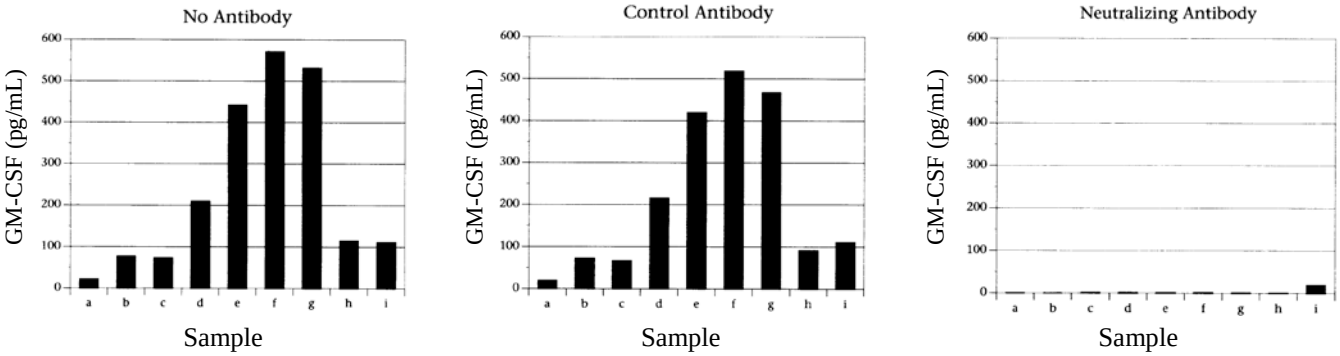
Calibration: The standards in this ELISA are calibrated to the NIBSC recombinant GM-CSF standard 88/646.
One (1) Pierce Endogen pg = 1 pg of NIBSC standard = 0.01 NIBSC units.



Expected Values: The average level of human GM-CSF found in 21 serum samples is 0.53pg/mL, ranging from 0 to 7.7pg/mL. The average level of human GM-CSF in 33 plasma samples is 2.5pg/mL, ranging from 0 to 48pg/mL. The average level of human GM-CSF found in 5 urine samples is 0.48pg/mL, ranging from 0 to 1.1pg/mL.

Linearity of Dilution: Linearity of dilution is determined by serially diluting six different positive samples. The dilutions are evaluated in the ELISA and the "found" doses are plotted against the "expected" doses.

Neutralization of Human GM-CSF "Positive Samples"
The specificity was confirmed by performing neutralization experiments. Nine human GM-CSF positive samples were incubated with no antibody, neutralizing polyclonal or a control IgG. These samples were then tested in the ELISA.



Cited Reference

1. *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds., 1987, Academic Press: New York, p71.

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Data Templates

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