



## Human GM-CSF ELISA Kit

Catalog Number: CRS-B007

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

**IMPORTANT: Please carefully read this user guide before performing your experiment.**

## Product information

This kit is specifically designed for the accurate quantitation of human GM-CSF from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

### NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

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## Contents

The kit contains sufficient reagents for 96 wells.

| Catalog    | Contents                                   | Amount  |
|------------|--------------------------------------------|---------|
| CRB007-C01 | Pre-coated Anti-GM-CSF Antibody Microplate | 1 plate |
| CRB007-C02 | Human GM-CSF Standard                      | 32 µg×2 |
| CRB007-C03 | Biotin-Anti-GM-CSF Antibody Con. Solution  | 100 µL  |
| CRB007-C04 | Biotin-Antibody Dilution Buffer            | 8 mL    |
| CRB007-C05 | Streptavidin-HRP Con. Solution             | 0.5 mL  |
| CRB007-C06 | Streptavidin-HRP Dilution Buffer           | 15 mL   |
| CRB007-C07 | 20× Washing Buffer                         | 50 mL   |
| CRB007-C08 | 1× Dilution Buffer                         | 15 mL×2 |
| CRB007-C09 | Substrate Solution                         | 12 mL   |
| CRB007-C10 | Stop Solution                              | 6 mL    |

**NOTE:** Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

## Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date. For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

| Contents                                      | Storage conditions                                                                                         |
|-----------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Pre-coated Anti-GM-CSF Antibody<br>Microplate | Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C. |
| Human GM-CSF Standard                         | Aliquot and store at -70°C<br>Avoid repeated freeze-thaw cycles.                                           |

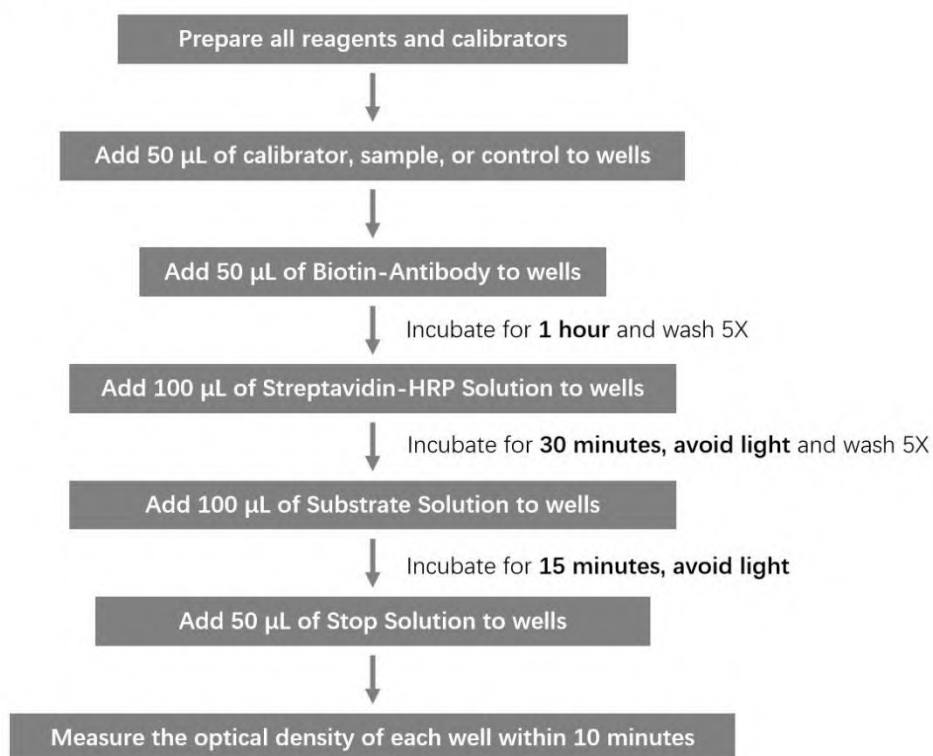
**NOTE:** Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

### Required materials not supplied.

|                    |                                                             |
|--------------------|-------------------------------------------------------------|
| <b>Instrument</b>  | Microplate reader capable of measuring absorbance at 450 nm |
| <b>Reagents</b>    | Deionized or distilled water                                |
| <b>Consumables</b> | 50 mL and 500 mL graduated cylinders                        |
|                    | Pipettes and pipette tips                                   |
|                    | Tubes to prepare standard dilutions.                        |

### Workflow

Analyte: GM-CSF



**NOTE:** Incubation temperature is 18 °C-25 °C

## Prepare the working buffers and standard dilutions.

**IMPORTANT:** Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

### Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-GM-CSF Antibody Solution: Add 60 µL of Biotin-Anti-GM-CSF Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. GM-CSF Streptavidin-HRP Solution: Add 160 µL of GM-CSF Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

### Prepare the reconstituted Standard.

Add 1.0mL ultrapure water to the provided lyophilized product (CRB007-C02) , dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted human GM-CSF Standard is 32 µg/mL.

**NOTE:** Avoiding vigorous shaking or vortexing. The reconstituted solution should be stored at -70°C. The freeze-thaw cycle should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.

### Prepare the standard serial dilutions.

1. Label a tube "Cm". Add 10 µL of the reconstituted human GM-CSF Standard and 990 µL of Sample Dilution Buffer to tube Cm, gently mix well.
2. Label 8 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8.
3. Add 5 µL of the liquid from **Cm** and 995 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =1600 pg/mL).
4. Prepare serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 800 pg/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-8 (12.5 pg/mL).
7. Sample Dilution Buffer serves as zero standard (blank).

## PROCEDURE OF ASSAY

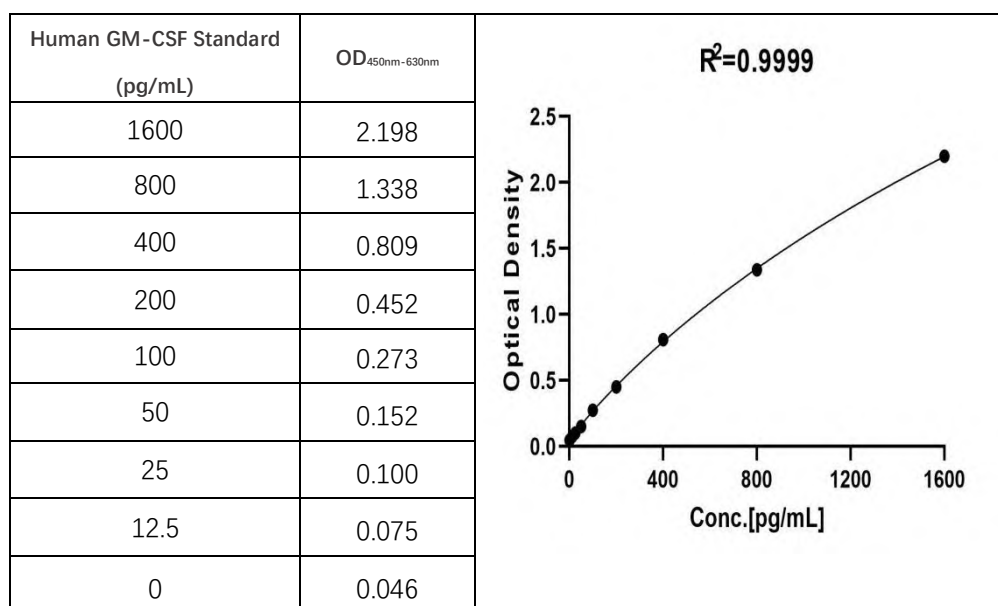
1. Add 50  $\mu$ L of GM-CSF Standard, sample, or control to wells.
2. Add 50  $\mu$ L Biotin-Anti-GM-CSF Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour**.
3. Aspirate each well and add 300  $\mu$ L of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
4. Add 100  $\mu$ L of GM-CSF Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light**.
5. Repeat step 3.
6. Add 100  $\mu$ L of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
7. Add 50  $\mu$ L of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.  
***Note:** the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.  
***Note:** To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

## CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero Standard (blank).
2. Establish a calibration curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Calibration curve:  $R^2 \geq 0.9900$ .
4. If the OD value of the sample to be tested is higher than the highest Standard, the sample shall be diluted with dilution buffer and assay repeated.

### Typical data

**Note:** For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.



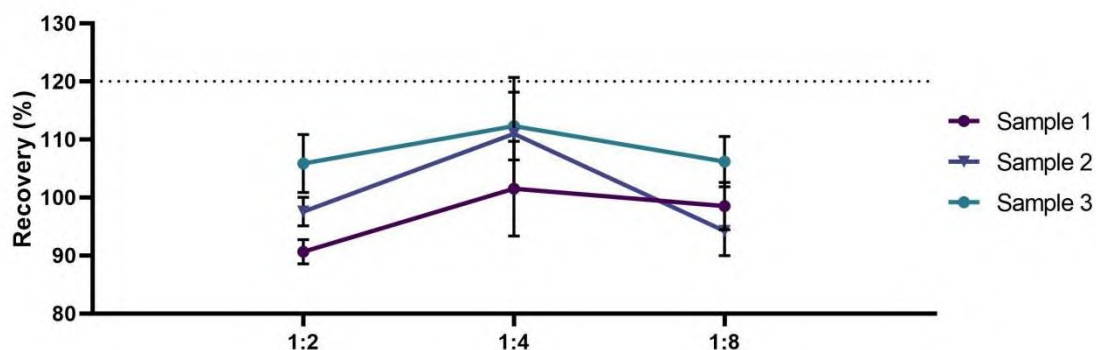
## PERFORMANCE CHARACTERISTICS

### 1. Sensitivity

The minimum detectable concentration (MDC) of GM-CSF is typically less than 10.0 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## 2. Linearity

Three samples (Serum) spiked with high concentrations of 2600 pg/mL, 2400 pg/mL and 2200 pg/mL were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of GM-CSF for serum samples is 101.98%.



## 3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different GM-CSF concentrations were tested in one assay. Acceptable criteria: CV < 10%.

| Sample Concentration (pg/mL) | Mean (pg/mL) | SD    | Numbers | CV (%) |
|------------------------------|--------------|-------|---------|--------|
| 1600                         | 1589.92      | 80.61 | 10      | 5.07   |
| 1200                         | 1227.07      | 60.37 | 10      | 4.92   |
| 800                          | 796.03       | 36.38 | 10      | 4.57   |

## 4. Inter-Assay Precision

Three samples containing different concentrations of GM-CSF were tested in independent assays. Acceptable criteria: CV<15%.

| Sample Concentration (pg/mL) | Mean (pg/mL) | SD    | Numbers | CV (%) |
|------------------------------|--------------|-------|---------|--------|
| 1600                         | 1534.90      | 78.43 | 9       | 5.11   |
| 1200                         | 1229.12      | 63.67 | 9       | 5.18   |
| 800                          | 809.82       | 34.74 | 9       | 4.29   |



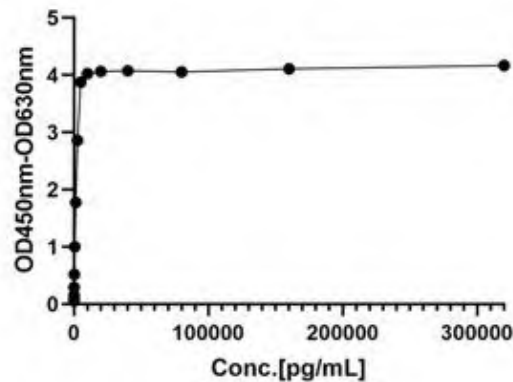
## 5. Recovery

Recombinant GM-CSF was spiked into 5 human serum samples, and then analyzed. The average recovery of GM-CSF for serum samples is 102.82%.

| Sample ID | Conc Measured (pg/mL) | Conc Added (pg/mL) | Conc Recovered (pg/mL) | Recovery (%) |
|-----------|-----------------------|--------------------|------------------------|--------------|
| 1         | 451.43                | 400                | 446.72                 | 111.68       |
|           | 314.05                | 300                | 309.34                 | 103.11       |
|           | 228.91                | 200                | 224.20                 | 112.10       |
|           | 4.96                  |                    |                        |              |
|           |                       |                    |                        |              |
| 2         | 378.91                | 400                | 375.36                 | 93.84        |
|           | 337.64                | 300                | 334.09                 | 111.36       |
|           | 218.05                | 200                | 214.49                 | 107.25       |
|           | 3.74                  |                    |                        |              |
|           |                       |                    |                        |              |
| 3         | 383.77                | 400                | 378.96                 | 94.74        |
|           | 338.49                | 300                | 333.68                 | 111.23       |
|           | 193.11                | 200                | 188.29                 | 94.15        |
|           | 5.07                  |                    |                        |              |
|           |                       |                    |                        |              |
| 4         | 392.88                | 400                | 390.84                 | 97.71        |
|           | 274.23                | 300                | 272.19                 | 90.73        |
|           | 226.99                | 200                | 224.95                 | 112.47       |
|           | 2.15                  |                    |                        |              |
|           |                       |                    |                        |              |
| 5         | 353.87                | 400                | 348.88                 | 87.22        |
|           | 330.65                | 300                | 325.66                 | 108.55       |
|           | 217.32                | 200                | 212.34                 | 106.17       |
|           | 5.25                  |                    |                        |              |

## 6. Hook Effect

Not bcted by the concentration of GM-CSF up to 80 ng/mL.



## 7. Interference Effect

To evaluate the hemolysis matrix effect and high-dose triglyceride matrix effect of assay, serum samples spiked with high concentrations of hemoglobin (2%), triglyceride (3 mg/mL), or Bilirubin (20 mg/dL) were tested. Results shown that all spiked analytes had recoveries between 85% and 120%, no hemolysis matrix effect and high-dose triglyceride matrix effect was observed in assay.

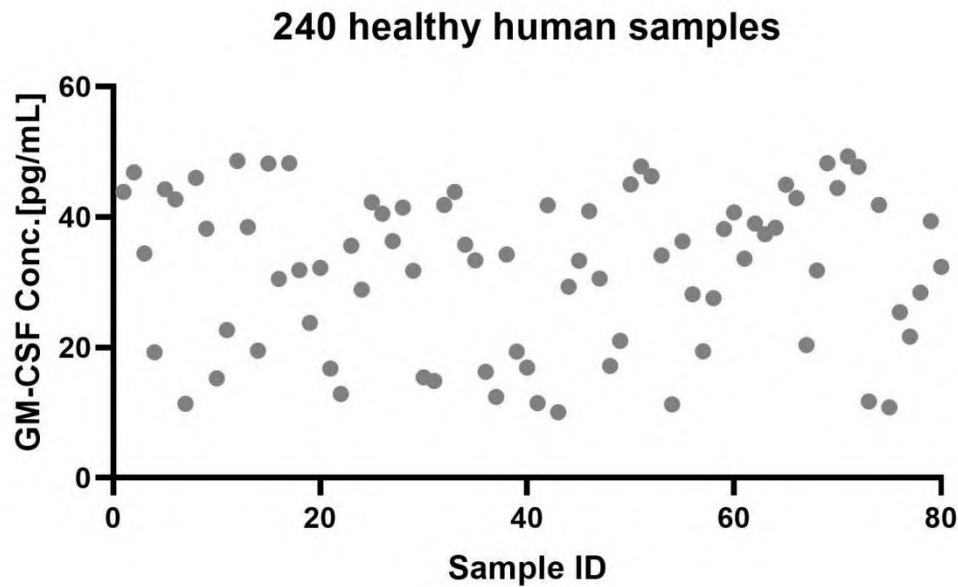
| Spiked Material     | ID              | Conc-1 (pg/mL) | Conc-2 (pg/mL) | Mean (pg/mL) | Recovery |
|---------------------|-----------------|----------------|----------------|--------------|----------|
| 2% Hemoglobin (v/v) | Sample 1        | 167.36         | 157.92         | 162.64       | 97%      |
|                     | Spiked Sample 1 | 152.80         | 164.16         | 158.48       |          |
|                     | Sample 2        | 3.09           | 3.07           | 3.08         | 99%      |
|                     | Spiked Sample 2 | 2.99           | 3.11           | 3.05         |          |

| Spiked material        | ID              | Conc-1 (pg/mL) | Conc-2 (pg/mL) | Mean (pg/mL) | Recovery |
|------------------------|-----------------|----------------|----------------|--------------|----------|
| Triglyceride (3 mg/mL) | Sample 1        | 152.16         | 154.56         | 153.36       | 104%     |
|                        | Spiked Sample 1 | 153.44         | 165.44         | 159.44       |          |
|                        | Sample 2        | 2.97           | 3.02           | 3.00         | 100%     |
|                        | Spiked Sample 2 | 3.07           | 2.91           | 2.99         |          |

| Spiked material      | ID              | Conc-1 (pg/mL) | Conc-2 (pg/mL) | Mean (pg/mL) | Recovery |
|----------------------|-----------------|----------------|----------------|--------------|----------|
| Bilirubin (20 mg/dL) | Sample 1        | 163.04         | 166.72         | 164.88       | 98%      |
|                      | Spiked Sample 1 | 158.56         | 166.24         | 162.40       |          |
|                      | Sample 2        | 2.85           | 3.12           | 2.99         | 100%     |
|                      | Spiked Sample 2 | 3.15           | 2.85           | 3.00         |          |

8. Sample Values

240 healthy serum samples were evaluated for the concentrations of human GM-CSF in assay.



9. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 1 µg/mL.

|       |                                                                                   |
|-------|-----------------------------------------------------------------------------------|
| Human | IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 p70, IL-10, MCP-1, M-CSF, TNF-α, IFN-γ |
|-------|-----------------------------------------------------------------------------------|

10. CALIBRATION

This immunoassay is calibrated against highly purified recombinant human GM-CSF produced at ACROBiosystems. The NIBSC/WHO International Standard for GM-CSF (88/646), which was intended as a potency standard, was evaluated in this kit. For conversion factors, contact tech support.

## TROUBLESHOOTING GUIDE

| Problem                                                            | Cause                                                            | Solution                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------|------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Poor standard curve</b>                                         | * Inaccurate pipetting                                           | * Check pipettes                                                                                                                                                                                                                                                          |
| <b>Large CV</b>                                                    | * Inaccurate pipetting<br>* Air bubbles in wells                 | * Check pipettes<br>* Remove bubbles in wells                                                                                                                                                                                                                             |
| <b>High background</b>                                             | * Plate is insufficiently washed<br>* Contaminated wash buffer   | * Review the manual for proper wash.<br>* Make fresh wash buffer                                                                                                                                                                                                          |
| <b>Very low readings across the plate</b>                          | * Incorrect wavelengths<br>* Insufficient development time       | * Check filters/reader<br>* Increase development time                                                                                                                                                                                                                     |
| <b>Samples are reading too high, but standard curve looks fine</b> | * Samples contain cytokine levels above assay range              | * Dilute samples and run again                                                                                                                                                                                                                                            |
| <b>Drift</b>                                                       | * Interrupted assay set-up<br>* Reagents not at room temperature | * Assay set-up should be continuous<br>- have all standards and samples prepared appropriately before commencement of the assay<br>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |