ClinMax™ 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.

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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.

Manufactured and distributed by
ACRODiagnostics Inc.
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Distributed by:
ACROBiosystems Inc.
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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	Sample Dilution Buffer	15 mL×2
CRB007-C09	Substrate Solution	12 mL
CRB007-C10	Stop Solution	6 mL

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.

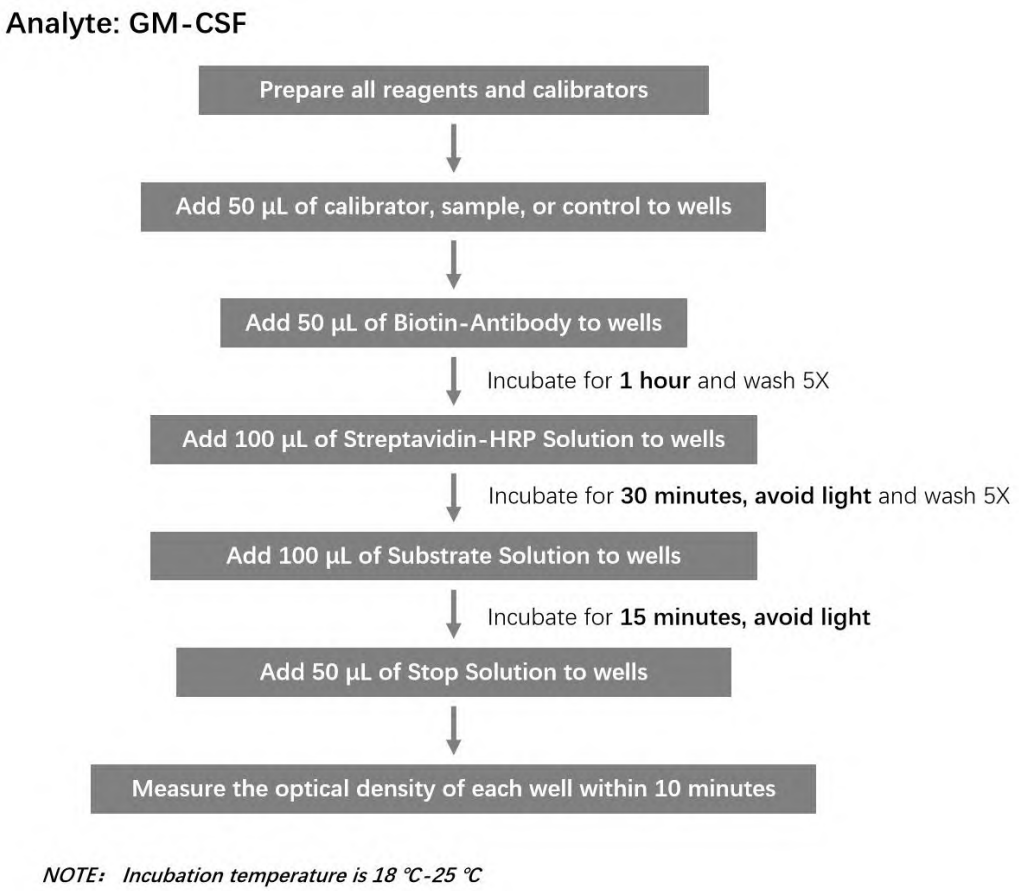
Contents	Storage conditions
Pre-coated Anti-GM-CSF Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human GM-CSF Standard	Aliquot and store at -70°C Avoid repeated freeze-thaw cycles.

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

Required materials not supplied.

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

Workflow



NOTE: Incubation temperature is 18 ℃-25 ℃

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 µL of GM-CSF Standard, sample, or control to wells.
2. Add 50 µL 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 hours.**
3. Aspirate each well and add 300 µL 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 µL of GM-CSF Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for 3**0 minutes, avoid light.**
5. Repeat step 3.
6. Add 100 µL 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 µL 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.*

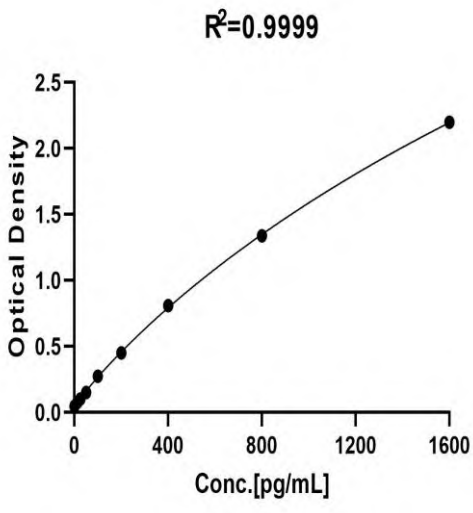
CALCULATION OF RESULTS

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: $R^2 \geqslant 0.9900$.
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.*

Human GM-CSF Standard (pg/mL)	$OD_{450nm-630nm}$
1600	2.198
800	1.338
400	0.809
200	0.452
100	0.273
50	0.152
25	0.100
12.5	0.075
0	0.046



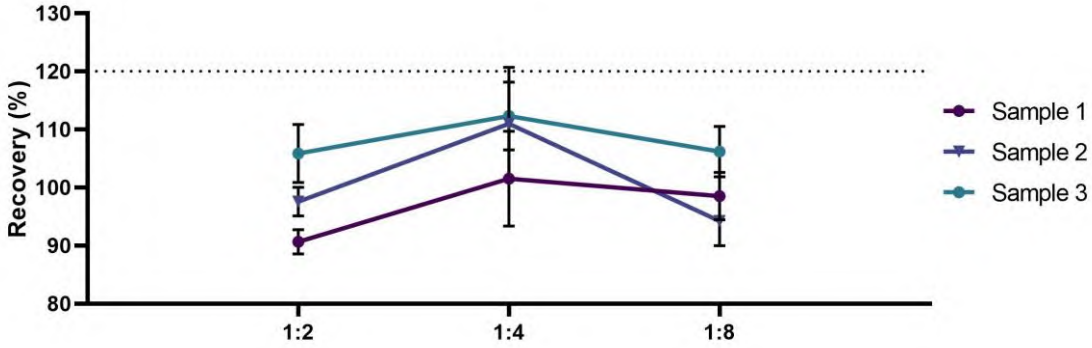
PERFORMANCE CHARACTERISTICS

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%

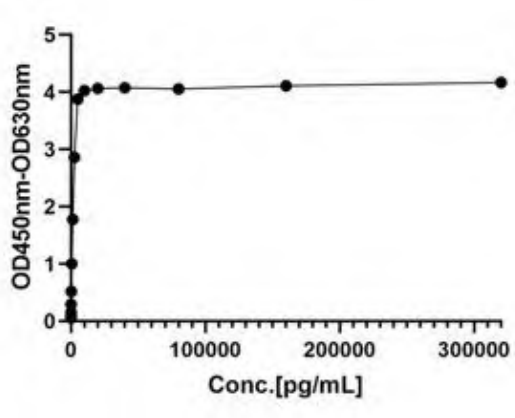
5. Recovery

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.

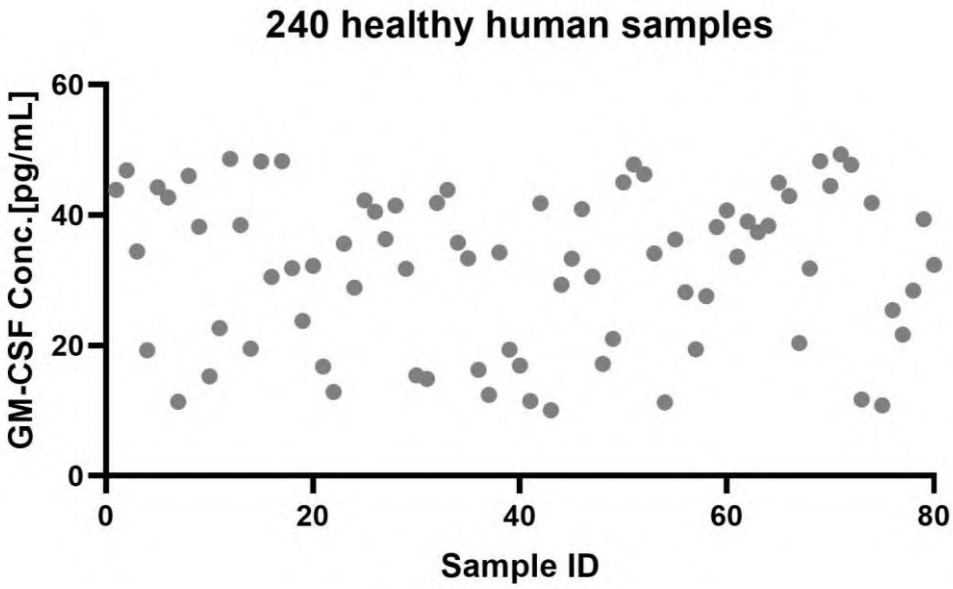
Spiked Material	ID	Conc-1 (pg/mL)	Conc-2 (pg/mL)	Mean (pg/mL)	Recovery
2% Hemoglobin (v/v)	Sample 1	167.36	157.92	162.64	97%
	Spiked Sample 1	152.80	164.16	158.48	
	Sample 2	3.09	3.07	3.08	99%
	Spiked Sample 2	2.99	3.11	3.05	

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.

TROUBLESHOOTING GUIDE

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