



ClinMax™ Human GM-CSF ELISA Kit, PRO

Catalog Number: CEA-C007

Pack Size: 96 tests

**IMPORTANT: Please carefully read this manual before performing your experiment.
For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure**

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INTENDED USE

This kit is specifically designed for the accurate quantitation of human Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) from serum and plasma. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the concentrations of human Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-GM-CSF Antibody. First add the standard samples provided in kit and your samples to the plate, next add detection antibody Biotin-Anti-GM-CSF Antibody to the plate, incubate and wash the wells. After wash add HRP-Streptavidin to the plate, incubate and wash the wells. Lastly load the substrate into the wells and color develops in proportion to the amount of GM-CSF bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm and 630nm.

LIMITATIONS OF THE PROCEDURE

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

MATERIALS PROVIDED

Table 1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CEA007-C01	Pre-coated Anti-GM-CSF Ab Microplate	1 plate	Solid	2-8 °C	2-8 °C
CEA007-C02	GM-CSF Calibrator	20µg ×2	Lyophilized powder	2-8 °C	-70 °C
CEA007-C03	Biotin-Anti-GM-CSF Ab Con. Solution	100 µL	Liquid	2-8 °C	2-8 °C
CEA007-C04	Biotin-Ab Dilution Buffer	8 mL	Liquid	2-8 °C	2-8 °C
CEA007-C05	GM-CSF SA-HRP Con. Solution	0.5 mL	Liquid	2-8 °C	2-8 °C
CEA007-C06	SA-HRP Dilution Buffer	15 mL	Liquid	2-8 °C	2-8 °C
CEA007-C07	20× Washing Buffer	50 mL	Liquid	2-30 °C	2-30 °C
CEA007-C08	Sample Dilution Buffer	15 mL ×2	Liquid	2-8 °C	2-8 °C
CEA007-C09	Substrate Solution	12 mL	Liquid	2-8 °C	2-8 °C
CEA007-C10	Stop Solution	6 mL	Liquid	2-30 °C	2-30 °C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Centrifuge;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized water / ultrapure water / distilled water to dilute 20× Washing Buffer.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g within 30 minutes of collection. Assay immediately.

Storage - Samples which cannot be assayed within 24 hours of collection should be frozen at -20°C or lower and will be stable for up to six months. Samples are allowed to freeze-thaw once.

KIT STORAGE AND EXPIRATION DATE

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (18-25 °C) before use. If crystals have formed in buffer solution, place the sample in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 µg.

Table 2. Preparation method

Catalog	Components	Size (96 tests)	Storage solution concentration	Reconstituted water volume
CEA007-C02	GM-CSF Calibrator	20 µg	20 µg/mL	1 mL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL 20×Washing Buffer with deionized water / ultrapure water / distilled water to 1000 mL.

1.2 Preparation of Biotin-Anti- GM-CSF Ab Solution

Prepare Biotin-Anti- GM-CSF Ab Solution by diluting 60 µL of Biotin-Anti- GM-CSF Ab Con. Solution into 6 mL Biotin-Ab Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

1.3 Preparation of GM-CSF SA-HRP Solution

Prepare GM-CSF SA-HRP Solution by diluting 160 µL of GM-CSF SA-HRP Con. Solution into 12 mL SA-HRP Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

2. Preparation of Calibration curve

The concentration of the reconstituted human GM-CSF Calibrator (CEA007-C02) is 20 µg/mL, prepare C_m by diluting 10 µL of the reconstituted human GM-CSF Calibrator into 990 µL Sample Dilution Buffer, mix gently well. Label 8 tubes, one for each standard point: C1, C2, C3, C4, C5, C6, C7, C8. According to the following dilution scheme: 5 µL GM-CSF C_m + 995 µL Sample Dilution Buffer. Shake gently to mix, labeled C1 ($C_1 = 1000$ pg/mL). Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 500 µL of Sample Dilution Buffer into each tube. Pipette 500 µL of diluted standard (concentration of standard = 1000 pg/mL) into the first tube, labeled C2 ($C_2 = 500$ pg/mL), and mix. Pipette 500 µL of this dilution into the second tube, labeled C3, and mix thoroughly before the next transfer. Sample Dilution Buffer serves as blank.

3. Add Samples and Biotin-Ab Solution

Add 50 µL GM-CSF Calibrator to each well, or add 50 µL samples to each well, finally add 50 µL Biotin-Anti- GM-CSF Ab Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for five times.

5. Add GM-CSF SA-HRP Solution

For all wells, add 100 µL GM-CSF SA-HRP Solution. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 30 min, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 15 min, avoid light.

8. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

9. Data Recording

Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

Note: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.

CALCULATION OF RESULTS

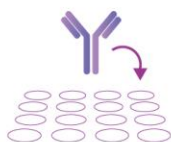
1. Normal range of Standard curve: $R^2 \geq 0.9900$.
2. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.
3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Log-Log Linear regression equation are used to draw the standard curve and calculate the sample concentration.

PRECAUTIONS FOR USE

1. All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
2. Do not use kit reagents beyond expiration date on label.
3. In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
4. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
5. Glass-distilled water or deionized water must be used for reagent preparation.
6. This kit should be used according to the provided instructions.
7. Do not mix reagents from different lots.
8. Bring all reagents and samples to room temperature (18-25 °C) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
9. This kit should be stored at 2-8 °C.

QUICK GUIDE

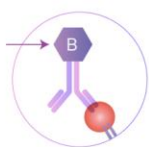
Quick Guide



1

Prepare

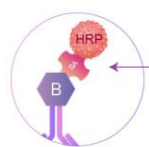
Prepare all reagents, standard curve, and samples as instructed.



2

Sample & detection antibody

Add test sample mix to wells. (Calibrator、samples、 Biotin-Ab Solution)

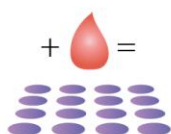


3

Streptavidin-HRP Solution

18-25°C 1.0 hour
Remove liquid and wash plate

Add enzyme conjugated Streptavidin



4

Substrate Reaction

18-25°C 30 min avoid light
Remove liquid and wash plate

Colorimetric substrate is added to the wells and will form a colored solution when catalyzed by the enzyme.



5

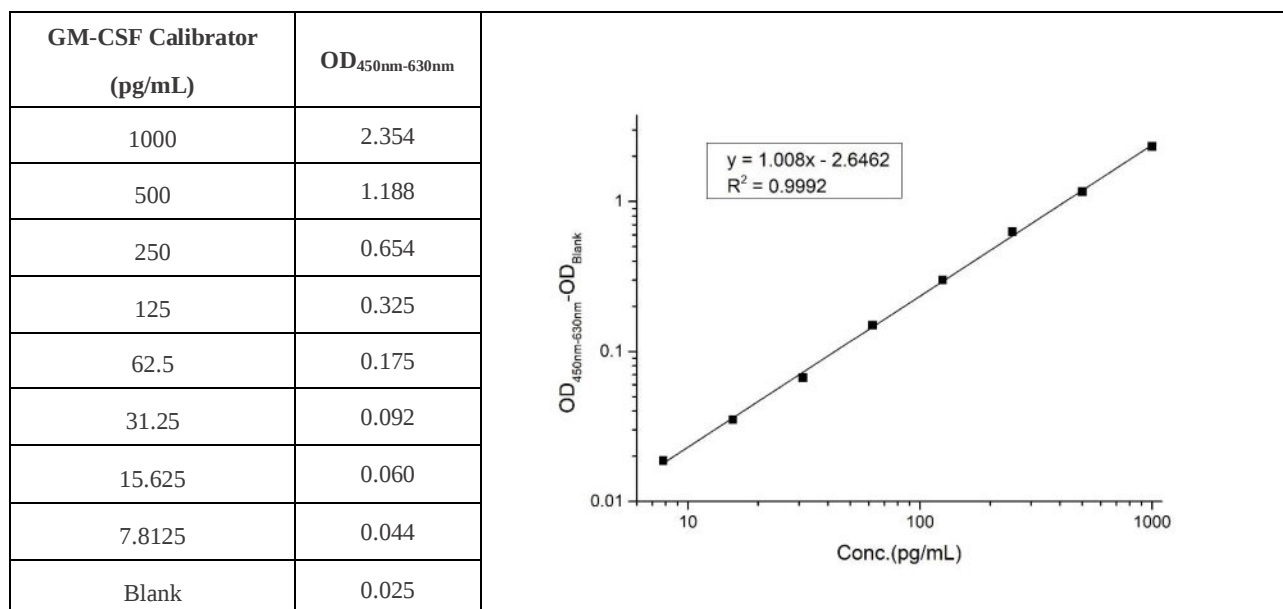
Termination +Analysis

18-25°C 15 min avoid light

Add Stop solution and read absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

TYPICAL DATA

Note: The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.



PERFORMANCE CHARACTERISTICS

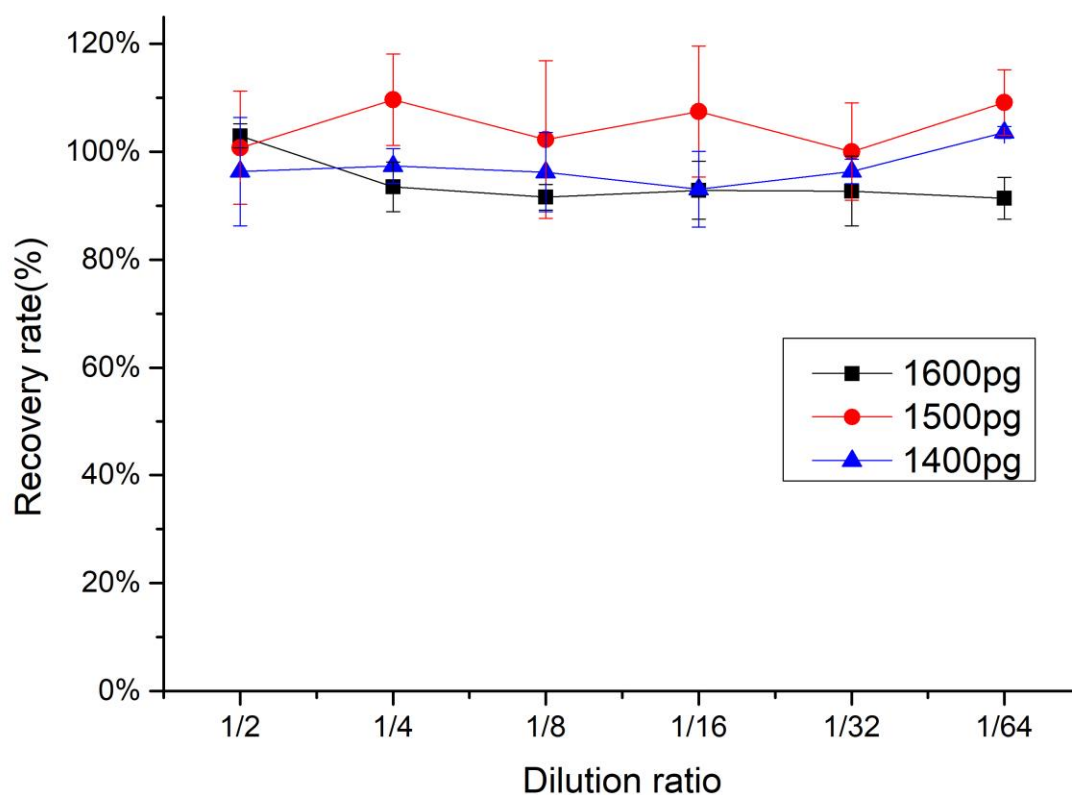
1. Sensitivity

The minimum detectable concentration of GM-CSF is less than 3.0 pg/mL.

	Lot 1	Lot 2	Lot 3
Number of Replicates	20	20	20
Limit of blank (pg/mL)	1.39	2.09	2.34

2. Linearity

Three human serum samples with high concentrations of GM-CSF were diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 with Dilution Buffer to produce samples with values within the dynamic range and then assayed. On average, 94.18% of GM-CSF was detected from serum samples



3. Calibration

This immunoassay has been calibrated against a highly purified human GM-CSF and is evaluated with standard from NIBSC/WHO. Reference Reagent GM-CSF (Human, rDNA derived) NIBSC code: 88/646.

NIBSC (88/646) approximate value (IU/mL) = $0.1 \times \text{ClinMax}^{\text{TM}}$ value (pg/mL).

4. 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-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p70, IL-13, IL-15, IL-17, TNF- α , IFN- γ
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5. Intra-Assay Statistics

Ten replicates of each of five samples containing different GM-CSF concentrations were tested in one assay , Intra-Assay Precision CV<10%.

Lot 1	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number of Replicates	10	10	10	10	10
Mean Concentration (pg/mL)	958.00	762.00	451.00	20.91	7.35
Standard Deviation	55.35	24.97	19.38	1.73	0.51
Coefficient of Variation (%)	5.78%	3.28%	4.30%	8.28%	6.87%
Lot 2	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number of Replicates	10	10	10	10	10
Mean Concentration (pg/mL)	763.20	679.20	405.60	16.95	6.17
Standard Deviation	41.60	44.04	16.21	1.08	0.46
Coefficient of Variation (%)	5.45%	6.48%	4.00%	6.38%	7.51%
Lot 3	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number of Replicates	10	10	10	10	10
Mean Concentration (pg/mL)	852.80	607.20	454.40	19.26	6.43
Standard Deviation	33.59	31.73	24.36	1.74	0.47
Coefficient of Variation (%)	3.94%	5.23%	5.36%	9.05%	7.34%

6. Inter-Assay Statistics

Five samples containing different concentrations of GM-CSF were tested in three independent assays, Inter-Assay Precision CV<15%.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Number of Assays	3	3	3	3	3
Mean Concentration (pg/mL)	858.00	682.80	437.00	19.04	6.65
Standard Deviation	43.51	33.58	19.98	1.52	0.48
Coefficient of Variation (%)	5.07%	4.92%	4.57%	7.98%	7.22%

7. Recovery

Recombinant GM-CSF was spiked into 5 human serum samples, and then analyzed. On average, 102.60% of GM-CSF was recovered from serum samples.

	Lot 1					Lot 2					Lot 3				
Sample Number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Spiked Volume	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sample volume	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
Sample Concentration (pg/mL)	4.96	3.74	5.07	2.15	5.25	4.96	3.74	5.07	2.15	5.25	4.96	3.74	5.07	2.15	5.25
Spiked Concentration(pg/ml)	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000	8000
Spiked Sample Concentration (pg/mL)	451.4 3	378.91	383.7 7	392.8 8	353.8 7	411.0 3	418.2 3	454.3 7	372.5 2	443.9 1	403.5 1	417.4 3	403.4 5	458.4 4	458.5 9
Recovery rate (%)	111.6 8%	93.84 %	94.74 %	97.71 %	87.22 %	101.5 8%	103.6 7%	112.3 9%	92.62 %	109.7 3%	99.70 %	103.4 7%	99.66 %	114.1 0%	113.4 0%
Spiked Concentration (pg/mL)	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000	6000
Spiked Sample Concentration (pg/mL)	314.0 5	337.64	338.4 9	274.2 3	330.6 5	295.6 3	281.3 0	295.1 9	304.7 2	303.9 5	279.4 1	345.5 6	324.2 3	311.4 6	303.9 3
Recovery rate (%)	104.3 3%	112.25 %	112.5 3%	91.10 %	109.9 4%	98.22 %	93.44 %	98.04 %	101.2 8%	100.9 7%	92.82 %	114.8 6%	107.7 6%	103.4 6%	100.9 5%

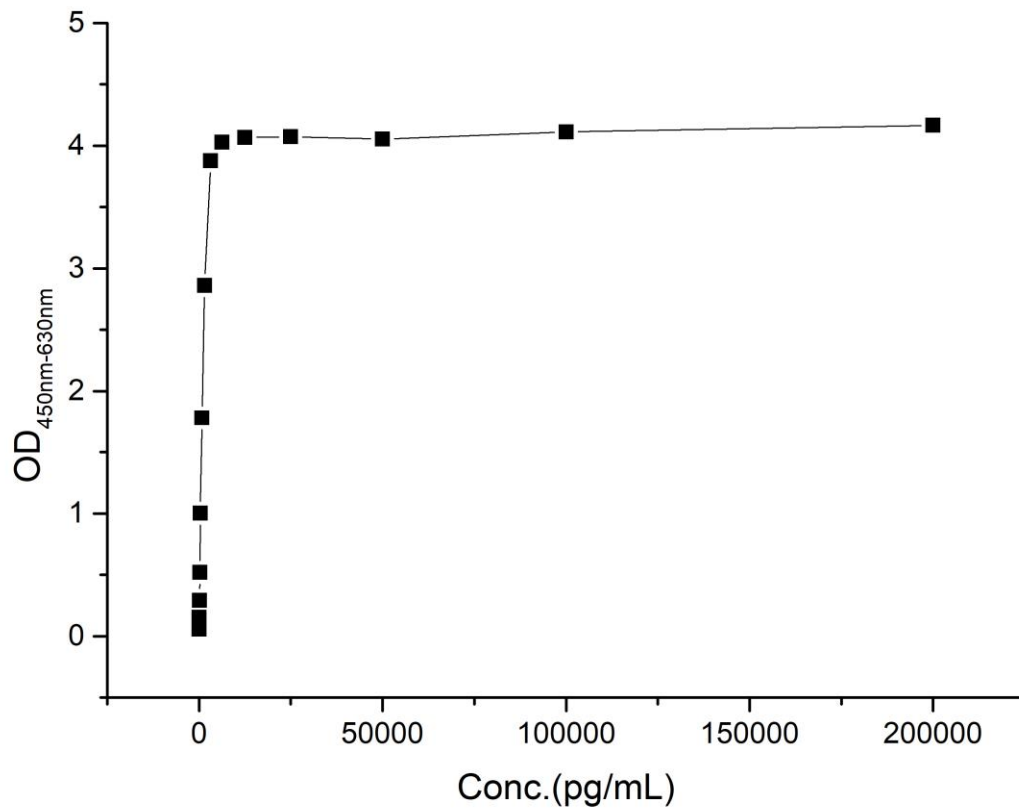
Spiked Concentration (pg/mL)	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000	4000
Spiked Sample Concentration (pg/mL)	228.9 1	218.05	193.1 1	226.9 9	217.3 2	194.2 1	185.4 3	204.4 5	189.7 6	207.3 2	206.7 4	228.3 5	189.8 4	198.5 2	204.5 2
Recovery rate (%)	113.9 6%	108.49 %	96.02 %	113.0 6%	108.1 4%	96.64 %	92.27 %	101.7 6%	94.40 %	103.1 8%	102.9 3%	113.6 3%	94.41 %	98.77 %	101.7 8%

Recombinant GM-CSF was spiked into cell supernatant sample, and then analyzed. On average, 97.73% of GM-CSF was recovered from serum samples.

	Lot 1	Lot 2	Lot 3
Spiked Volume	1	1	1
Sample volume	19	19	19
Sample Concentration (pg/mL)	5.55	2.12	4.02
Spiked Concentration(pg/ml)	9000	9000	9000
Spiked Sample Concentration (pg/mL)	497.40	479.33	453.14
Recovery rate (%)	109.36%	106.07%	99.85%
Spiked Concentration (pg/mL)	7000	7000	7000
Spiked Sample Concentration (pg/mL)	319.36	322.27	314.30
Recovery rate (%)	90.95%	91.79%	89.53%
Spiked Concentration (pg/mL)	5000	5000	5000
Spiked Sample Concentration (pg/mL)	241.16	250.42	241.10
Recovery rate (%)	96.12%	99.82%	96.10%

8. Hook Effect

Not be affected by GM-CSF concentrations up to 10 ng/mL.



9. Interference effect

Bilirubin (simulated jaundice) concentration should be less than 20mg/dL, hemoglobin (simulated hemolysis) concentration should not be higher than 3500mg/dL, triglyceride (simulated lipemia) concentration may not be higher than 2.0g/L, Heparin concentration should be less than 40U/mL, EDTA concentration should be less than 4mg/mL, and Sodium citrate concentration may not be higher than 40mg/mL, it does not affect the detection result.

	Bilirubin (20mg/dL)	Hemoglobin (3500mg/dL)	Triglyceride (2.0g/L)	Heparin (40U/mL)	EDTA Plasma (4mg/mL)	Sodium citrate Plasma(40mg/mL)
Spiked Sample Concentration (pg/mL)-1	105.23	98.50	102.28	104.18	103.44	102.39
Sample Concentration (pg/mL)-1	108.07	107.06	101.16	98.97	106.65	95.02
RD	-2.70%	-8.70%	1.10%	5.00%	-3.10%	7.20%
Spiked Sample Concentration (pg/mL)-2	1.84	1.78	1.86	1.70	1.79	1.70
Sample Concentration (pg/mL)-2	1.54	1.49	2.18	1.88	2.11	1.50
Results	≤Lob	≤Lob	≤Lob	≤Lob	≤Lob	≤Lob

10. Sample values

240 human healthy sample were evaluated for the presence of human GM-CSF in this assay. 107 samples measured less than the lowest standard, 3 pg/mL. 133 samples measured between 3 and 1000 pg/mL.

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