Human G-CSF ELISA Kit

Catalog Number：CEA-C221

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 G-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.
TEL: 010-67855298-8147

Distributed by:
ACROBiosystems Inc.
US & Canada TEL: +1 800-810-0816
Asia & Pacific TEL: +86 400-682-252

Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA221-C01	Pre-coated Anti-G-CSF Antibody Microplate	1 plate
CEA221-C02	Human G-CSF Standard	15 µg
CEA221-C03	Biotin-Anti-G-CSF Antibody	20 µg
CEA221-C04	Streptavidin-HRP	50 µL
CEA221-C05	10×Washing Buffer	50 mL
CEA221-C06	2×Dilution Buffer	50 mL
CEA221-C07	Substrate Solution	12 mL
CEA221-C08	Stop Solution	7 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-G-CSF Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.
Human G-CSF Standard	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.
Biotin-Anti-G-CSF Antibody	Aliquot and store for up to 1 month at -70°C in a freezer. Avoid repeated freeze-thaw cycles.

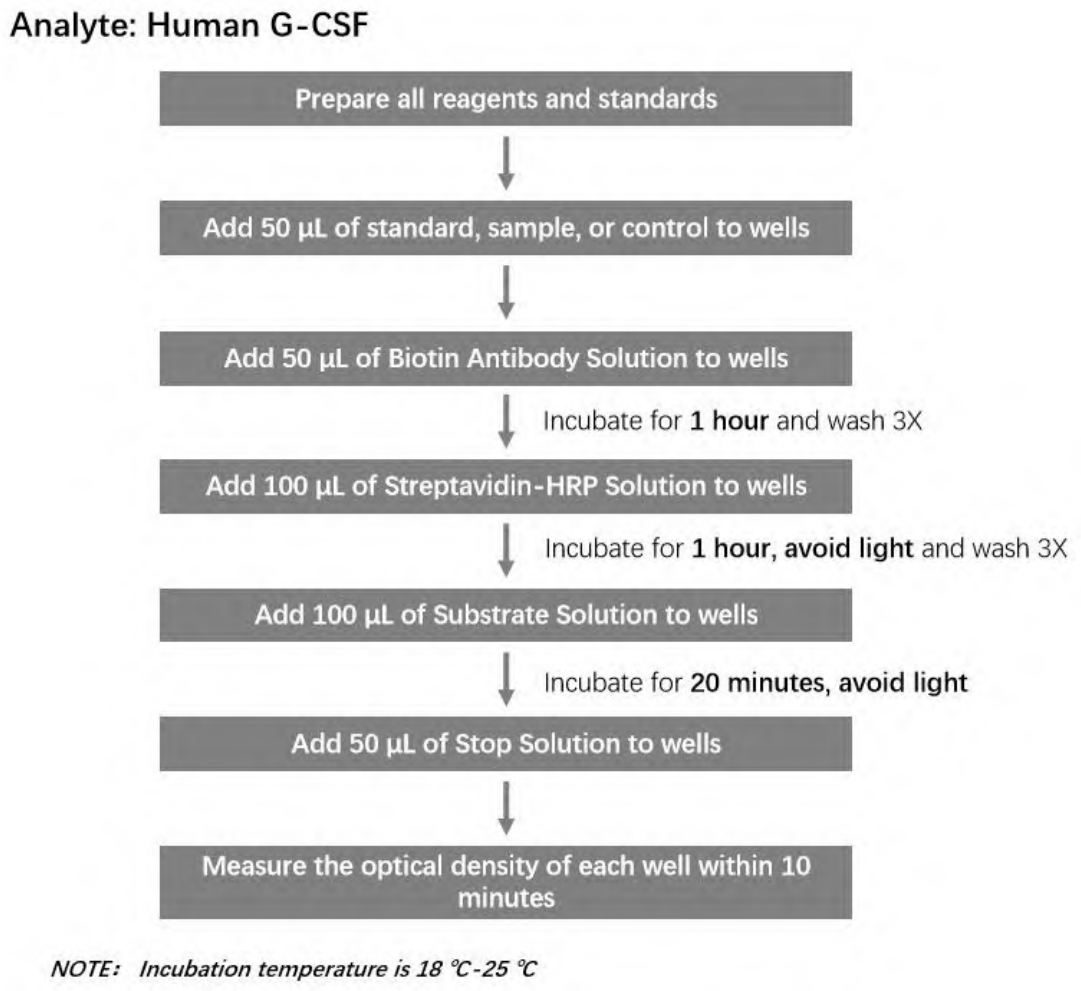
NOTE: Streptavidin-HRP and Substrate Solution should avoid light.

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.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure 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.

Prepare the reconstituted lyophilized product.

1. Add 100 µL ultrapure water to the provided lyophilized product（CEA221-C02）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted Human G-CSF Standard is 150 µg/mL.
2. Add 100 µL ultrapure water to the provided lyophilized product（CEA221-C03）, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted Biotin-Anti-G-CSF Antibody is 200 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.*

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 10×Washing Buffer with deionized or distilled water to 500 mL.
2. 1×Dilution Buffer: Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.
3. Biotin-Anti-G-CSF Antibody Solution: Dilute reconstituted Biotin-Anti-G-CSF Antibody （200 µg/mL） to 0.4 µg/mL with 1×Dilution Buffer. The solution was freshly prepared just before use.
4. Streptavidin-HRP Solution: Add 6 µL of Streptavidin-HRP to 12 mL of 1×Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the standard serial dilutions.

1. Label a tube "**Cm-0**". Add 10 µL of the reconstituted human G-CSF Standard and 990 µL of 1×Dilution Buffer to tube Cm-0, gently mix well.
2. Label a tube "**Cm-1**". Add 10 µL of the **Cm-0** and 990 µL of 1×Dilution Buffer to tube Cm-1, gently mix well.
3. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
4. Add 30 µL of the liquid from **Cm-1** and 1095 µL of 1×Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =400 pg/mL).
5. Prepare 1:1 serial dilutions for the standard curve as follows: Add 500 µL of 1×Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
6. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 200 pg/mL).
7. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
8. 1×Dilution Buffer serves as zero standard (blank).

PROCEDURE OF ASSAY

1. Add 50 µL of G-CSF Standard, sample, or control to wells.
2. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour.**
3. Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, 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 three times.
4. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour, 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 **20 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 10 minutes.
   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

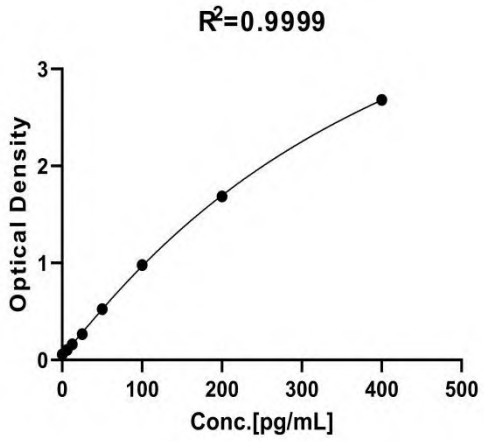
CALCULATION OF RESULTS

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 standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard 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 G-CSF Standard(pg/mL)	$OD_{450nm-630nm}$
400	2.681
200	1.686
100	0.979
50	0.524
25	0.266
12.5	0.161
6.25	0.103
Blank	0.056



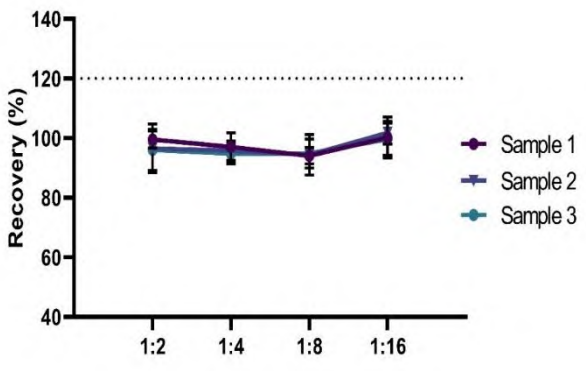
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of G-CSF is typically less than 6.25 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 G-CSF 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 G-CSF for serum samples is 97.1%.



3. Intra-Assay Precision

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

Sample Concentration (pg/mL)	Mean (pg /mL)	SD	Numbers	CV (%)
300	286	7.26	10	2.5
200	186	5.33	10	2.9
12	12.0	1.04	10	8.6

4. Inter-Assay Precision

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

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
300	290	7.60	9	2.6
200	191	10.8	9	5.7
12	12.7	0.884	9	7.0

5. Recovery

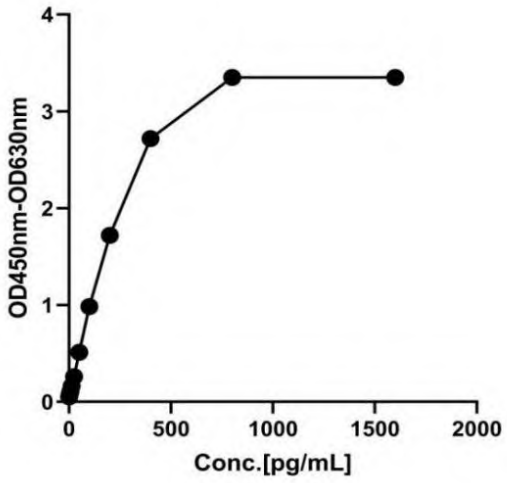
Recombinant G-CSF was spiked into 3 human serum samples, and then analyzed. The average recovery of G-CSF for serum samples is 101.2.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	299	300	298	99.2
	201	200	200	99.9
	15.1	12	13.7	113.9
	1.58	-		
2	278	300	277	92.4
	188	200	187	93.7
	14.2	12	13.6	113.1
	0.746	-		
	285	300	283	94.5

3	185	200	184	92.1
	14.6	12	13.5	112.1
	1.23	-		

Hook Effect

Not be affected by the concentration of G-CSF up to 1600 pg/mL.



6. Sample Values

Serum: 30 healthy donor serum samples were evaluated for the concentrations of human G-CSF in assay. The sample detection rate is 36.7%. The range of detected samples is 100.46 pg/mL-55802.16 pg/mL with a mean of 10352.50 pg/mL.

Cell Culture Supernates: PBMC (5.0×10^6cells/mL) were cultured in 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 ug/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 0.1ug/mL LPS for 2 days or 5 days. Subsequent analysis involved quantitative measurement of human G-CSF concentrations in collected culture supernatants using standardized immunoassays.

Condition	Day 2 Mean (pg/mL)	Day 5 Mean (pg/mL)
Unstimulated	1466	1042
Stimulated with LPS	4759	7773

7. Specificity

No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines at up to 25.6 ng/mL.

Human	M-CSF、GM-CSF
Mouse	GM-CSF

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