

Human G-CSF ELISA Kit

Catalog Number KHC2031 (96 tests) and KHC2032 (2 × 96 tests)

Pub. No. MAN0014765 Rev. 2.0 (30)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human G-CSF ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of human G-CSF in human serum, plasma, buffered solution, or cell culture medium. . The assay recognizes both natural and recombinant human G-CSF.

G-CSF (granulocyte colony-stimulating factor) is secreted as a glycoprotein monomer containing 174 amino acids that support growth of hematopoietic colonies. It consists of four α-helical bundle structures, and amino acid residues from 20 to 46, and the carboxy terminus are important for binding to its receptor.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHC2031 (96 tests)
Hu G-CSF Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Plate; 96-well strip-well plate	1 plate
Hu G-CSF Biotin Conjugate; contains 0.1% sodium azide	6 mL
Streptavidin-HRP (100X); contains 3.3 mM thymol	0.125 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer—automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at thermofisher.com for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

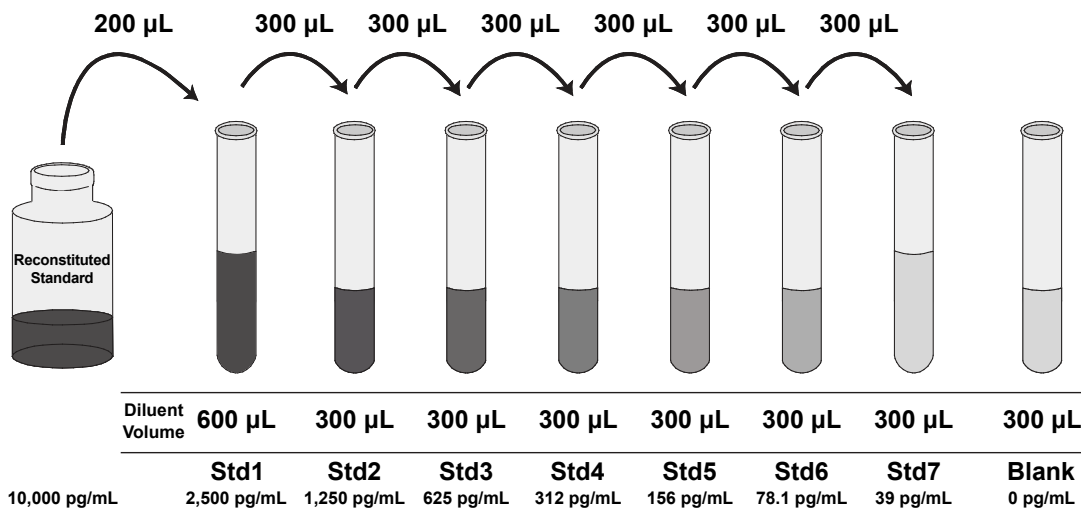
Perform sample dilutions with Standard Diluent Buffer

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This assay has been calibrated against the WHO reference preparation 88/502 (version 4). One nanogram equals 600 International Units.

1. Reconstitute Hu G-CSF Standard to 10,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 10,000 pg/mL human G-CSF. **Use the standard within 1 hour of reconstitution.**
2. Add 200 μ L Reconstituted Standard to one tube containing 600 μ L Standard Diluent Buffer and mix. Label as 2,500 pg/mL human G-CSF.
3. Add 300 μ L Standard Diluent Buffer to each of 7 tubes labeled as follows: 1,250, 625, 312, 156, 78.1, 39, and 0 pg/mL human G-CSF.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard all remaining reconstituted and diluted standards after completing assay. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 μ L Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

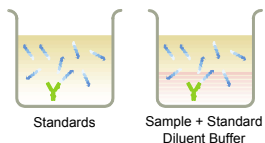
Perform ELISA (Total assay time: [3 hours])

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.



1 Bind antigen



- Add 100 μL of standards to the appropriate wells. For serum, plasma, buffered solution, cell culture, and controls, add 50 μL of Standard Diluent Buffer followed by 50 μL of sample (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
- Add 50 μL Hu G-CSF Biotin Conjugate solution into each well except the chromogen blanks.
- Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at 37°C.
- Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Streptavidin-HRP



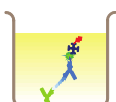
- Add 100 μL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
- Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
- Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

3 Add Stabilized Chromogen



- Add 100 μL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
 - Incubate for 30 minutes at room temperature in the dark.
- Note:** TMB should not touch aluminum foil or other metals.

4 Add Stop Solution



Add 100 μL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 2,500 pg/mL human G-CSF.

Standard Human G-CSF (pg/mL)	Optical Density (450 nm)
2500	2.77
1250	1.63
625	0.96
312	0.56
156	0.34
78.1	0.23
39.0	0.19
0	0.16

High-dose hook effect

Samples spiked with human G-CSF up to 1.5 mg/mL gave responses higher than that obtained for the last standard point.

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	273	547	778
Standard Deviation	22.6	49.9	66.9
% Coefficient of Variation	8.3	9.1	8.6

Intra-assay precision

Samples of known human G-CSF concentration were assayed in replicates of 12 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	250	591	757
Standard Deviation	12.9	41.0	53.3
% Coefficient of Variation	5.2	6.9	7.0

Expected values

Twenty serum and twenty plasma (EDTA) samples were evaluated in this assay. All samples measured <39 pg/mL (the lowest concentration in the human G-CSF standard curve).

Linearity of dilution

Human serum and cell culture medium containing 10% fetal calf serum were spiked with human G-CSF and serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (pg/mL)	Expected (pg/mL)	%	Measured (pg/mL)	Expected (pg/mL)	%
Neat	2,148	—	—	1,942	—	—
1/2	911	1,074	85	996	971	103
1/4	460	537	86	479	486	99
1/8	310	269	115	223	243	92
1/16	124	134	93	122	121	101

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Recovery

The recovery of human G-CSF added to human serum averaged or to cell culture medium containing fetal bovine serum (FBS) was measured with the Human G-CSF ELISA Kit.

Sample	Range %	Average % Recovery
Serum (n=8)	70-92	83
Cell culture medium + 1% (FBS)	—	108
Cell culture medium + 10% (FBS)	—	113








Sensitivity

The analytical sensitivity of the assay is <20 pg/mL human G-CSF. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Specificity

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the Human G-CSF ELISA Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-1 β , IL-2, sIL-2R, IL-3, IL-4, IL-5, sIL-6R, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-16, GM-CSF, IFN- γ , TNF- α ; **mouse** IL-3, G-CSF, GM-CSF.

Product label explanation of symbols and warnings

 REF	Catalog Number	 LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents
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Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

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