Human G-CSF ELISA Kit

Catalog Number
EK0463

For the quantitative determination of human granulocyte colony stimulating factor (G-CSF) concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

Contact information

Tel: 1-301-446-2499 Fax: 1-301-446-2413

Email: tech@signalwayantibody.com Web: www.sabbiotech.com

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for G-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any G-CSF present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for G-CSF is added to the wells and binds to the combination of capture antibody- G-CSF in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of G-CSF present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven G-CSF standard dilutions and G-CSF sample concentration determined.

DETECTION RANGE

7.8 pg/ml - 500 pg/ml

SENSITIVITY

The minimum detectable dose was 4pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human G-CSF. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 1: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Other proteins
EGF	FGF-8b	bovine FGF acidic
GM-CSF	FGF-8c	bovine FGF basic
FGF-4	G-CSF	human PDGF
FGF-5	M-CSF	porcine PDGF
FGF-6	VEGF-120	
FGF-9□	VEGF-164	
FGF-10		

PRECISION

The coefficient of variation of both intra-assay and inter-assay were less than 10%.

MATERIALS PROVIDED

1. Aluminium pouches with a Microwell Plate coated with antibody to human G-CSF (8×12)
2. 2 vials human G-CSF Standard lyophilized, 2000 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-human G-CSF antibody
4. 2 vials Streptavidin-HRP solution
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 4 pieces Adhesive Films
12. package insert

STORAGE

Table 2: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="8">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at ≤20℃.</td></tr>
</table>

		Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**

**Provided this is within the expiration date of the kit.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.

9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 2000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 750μL of Standard/sample Diluent into the 500 pg/mL tube and pipette 500μL of Standard/sample Diluent into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human G-CSF antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

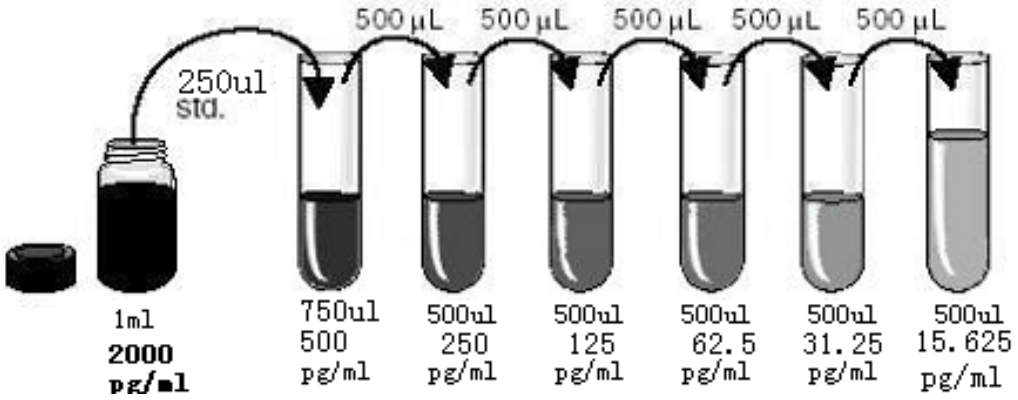

Figure 1: Preparation of G-CSF standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for

running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.

3. Add 100μL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 μL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 3.
7. Add 100 μL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 3.
9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

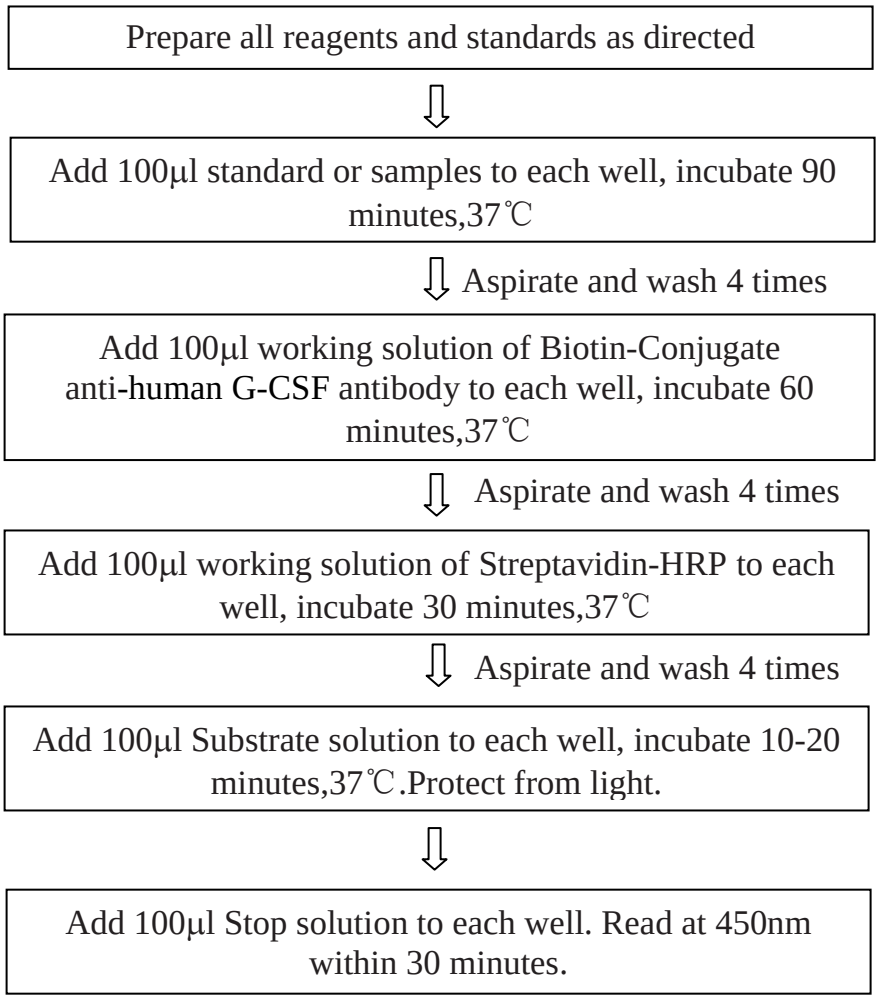

Figure 2: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the G-CSF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3:Typical data using the G-CSF ELISA (Measuring wavelength:450nm,Reference wavelength:630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.080	0.086	0.083	——
7.8	0.190	0.198	0.195	0.181
15.625	0.256	0.253	0.255	0.242
31.25	0.366	0.376	0.371	0.361
62.5	0.590	0.597	0.594	0.588
125	1.006	0.998	1.002	1.002
250	1.659	1.662	1.661	1.668
500	2.357	2.353	2.355	2.353

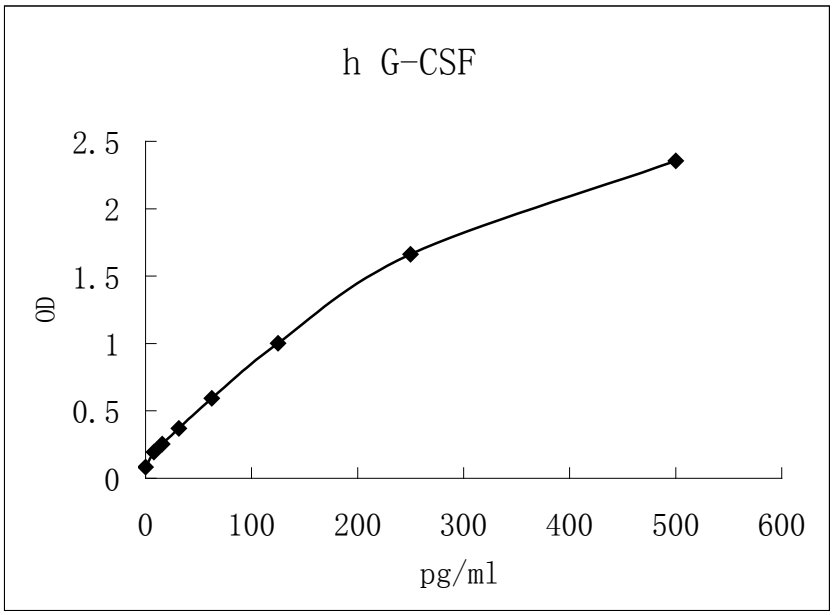

Figure 3:Representative standard curve for G-CSF ELISA. G-CSF was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

BACKGROUND

G-CSF is a pleiotropic cytokine best known for its specific effects on the proliferation, differentiation, and activation of hematopoietic cells of the neutrophilic granulocyte lineage. It is produced byactivated monocytes/macrophages, fibroblasts, endothelial cells, astrocytes, osteoblasts and bone marrow stromal cells. In addition, various carcinoma cell lines and myeloblastic leukemia cells can produce G-CSF constitutively. G-CSF and G-CSF receptors have also been shown to be produced by placental, decidual and endometrial gland cells during pregnancy, suggesting that G-CSF may play a role in decidual and placental functions (1). Clinical use of G-CSF has been approved for the amelioration of chemotherapy-induced neutropenia as well as for severe chronic neutropenia following bone marrow transplants. Several reviews on G-CSF and its clinical applications have been published (2 - 6). In humans, two

distinct cDNA clones for G-CSF, encoding 207 and 204 amino acid (aa) residue precursor proteins, have been isolated. Both proteins have a 30 aa signal peptide and have identical sequences except for a three amino acid insertion (deletion) at the 35th aa residue from the N-terminus of the mature protein. The 177 aa residue G-CSF, derived from the 207 aa residue precursor, was reported to have a ten fold lower specific activity than the shorter G-CSF isoform. Human G-CSF is 73% identical at the amino acid level to mouse G-CSF and the two proteins show species cross-reactivity (3, 4). G-CSF exerts its biological effects through binding to specific cell surface receptors. A single class of high affinity binding sites is present in both human and mouse hematopoietic progenitors and neutrophilic granulocytes. Among nonhematopoietic cells, receptors are also expressed on placental cells, endothelial cells, and various carcinoma cell lines. cDNAs for the human and mouse G-CSF receptors have been isolated (7 - 10). Mature mouse or human G-CSF receptors are single transmembrane glycoproteins of approximately 800 aa residues. The extracellular domain of the G-CSF receptor contains three regions of homology with other cell surface proteins, including an N-terminal immunoglobulin-like region, a structural motif characteristic of the hematopoietin receptor superfamily, and three tandem repeats of the fibronectin type III domain. In humans, four alternative transcripts of the G-CSF receptor gene, encoding a soluble form of G-CSF receptor as well as three transmembrane receptor isoforms have been identified (7).

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