

Rat Anti-Human Granulocyte-Colony Stimulating Factor (G-CSF) ELISA Set

<u>Cat. No.</u>	<u>Form</u>	<u>Quantity</u>
15450-01	ELISA Set	1

For Research Use Only

Intended Use

This set is specifically designed for quantitative determination of human Granulocyte-Colony Stimulating Factor (G-CSF) concentrations in cell culture supernatant, plasma and serum.

Background

Granulocyte-Colony Stimulating Factor (G-CSF) is a 17kD glycoprotein that is primarily secreted by monocytes and macrophages, but is also secreted by endothelial cells and fibroblasts. G-CSF receptors are localized to hematopoietic cells with neutrophil-granulocyte lineage and upon ligand binding induce signaling through the Jak/Stat pathway¹. This pleiotropic cytokine promotes the proliferation, differentiation and function of neutrophil lineage committed cells and therefore plays an intricate role in pathogen defense, inflammation and tissue repair^{2,3}. Clinically, G-CSF is used to ameliorate the symptoms of chemotherapy-induced neutropenia^{4,5}.

Principle of Assay

This assay incorporates a quantitative sandwich enzyme immunoassay technique. Unlabeled monoclonal antibody specific for human G-CSF is first coated onto 96 well microplate(s). Standards and samples are then added to the wells and any G-CSF present is captured by the immobilized antibody. After a wash to remove unbound material, biotin-labeled anti-human G-CSF detection antibody is added to the wells, followed by a horseradish peroxidase-labeled anti-biotin antibody incubation. After another wash, TMB substrate solution is added that will result in a blue color proportional to the amount of bound G-CSF. Color development is then quenched and intensity is measured at 450nm.

Research Applications

Enzyme-Linked-Immunosorbent-Assay (ELISA)

Characterization

To ensure lot-to-lot consistency, each batch of product is tested by ELISA to conform to the characteristics of a standard reference reagent.

Assay Kit Components

- 0.3mL G-CSF Capture Antibody: unlabeled, 200X
- 0.06mL G-CSF Detection Antibody: biotin-conjugated, 1000X
- 0.3mL Revealing Antibody: HRP-conjugated, 200X

- 2 vials Standard: lyophilized recombinant human G-CSF, 20ng/vial
- 15mL Coating Buffer (5X)
- 300mL Assay Diluent (1X)
- 300mL Washing Buffer (20X)
- 60mL Standard/Sample Diluent (1X)
- 60mL TMB one component microwell substrate
- 60mL TMB Stop Solution
- 5 x 96-well ELISA plates

Sample Collection and Storage

Cell Culture Supernatant - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using citrate, EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Plasma and serum samples require a 200-fold dilution. A suggested 200-fold dilution is 5.0µL sample into 995µL Assay Diluent.

Reagent Preparation

All reagents should be diluted immediately prior to use. Do not add sodium azide to Assay Diluent as it inhibits the activity of horseradish-peroxidase. It is recommended that all samples, controls and standards be assayed in duplicate or triplicate.

- Dilute 5X Coating Buffer to 1X with distilled (DI) water. A suggested dilution (for one plate) consists of 2.2mL 5X Coating Buffer with 8.8mL of DI water.
- Dilute unlabeled human G-CSF specific Capture Antibody 200-fold with 1X Coating Buffer. A suggested dilution (for one plate) consists of 55µL Capture Antibody with 10.945mL 1X Coating Buffer.
- Dilute 20X Wash Buffer to 1X with DI water. A suggested dilution consists of 50mL 20X Wash Buffer with 950mL of DI water.
- Reconstitute 1 vial lyophilized human G-CSF Standard by adding 0.1mL DI water to 1 vial (200ng/mL) and gently vortex. Next, dilute G-CSF Standard 100-fold by adding 5µL 200ng/mL Standard to 495µL DI water to yield a final concentration of 2000pg/mL and allow the standard to equilibrate for at least 15 minutes. Aliquot any unused standard into polypropylene vials and store at -80°C.
- Dilute biotin-labeled human G-CSF specific Detection Antibody 1000-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 11µL Detection Antibody with 10.989mL 1X Assay Diluent.
- Dilute horseradish peroxidase-labeled Revealing Antibody 200-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 55µL Revealing Antibody with 10.945mL 1X Assay Diluent.

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Corporate Offices: 160A Oxmoor Blvd. • Birmingham, AL 35209 • **USA Mailing Address:** P.O. Box 26221 • Birmingham, AL 35260 • USA
Tel: 205.945.1774 • U.S. and Canada: 800.722.2255 • **Fax:** 205.945.8768
Email: info@southernbiotech.com • **Website:** www.southernbiotech.com

Assay Procedure

1. Collect samples and prepare reagents as directed previously.
2. Add 100µL of diluted Capture Antibody solution to all wells of a provided 96-well plate. Incubate at 4°C overnight.
3. Wash entire plate 4 times with 1X Wash Buffer.
4. Add 300µL of 1X Assay Diluent per well to block non-specific binding and reduce background. Incubate at room temperature for 30 minutes.
5. Wash plate as in Step 3.
6. Prepare standard dilution by adding 100µL/well of appropriate dilution to the plate. To do this, perform six two-fold serial dilutions of the 2000pg/mL top standard either within the plate or in separate tubes. To dilute within the plate, add 200µl of 2000pg/mL human G-CSF Standard to row A and 100µl Standard/Sample Diluent to rows B through H. Perform serial dilution by taking 100µl standard from row A and mix with row B. Then take 100µl from row B and add it to row C and continue for each subsequent row. Therefore, the final human G-CSF standard concentrations will be 2000pg/mL, 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL and 31.3pg/mL. Standard/Sample Diluent alone serves as the zero standards for row H (0pg/mL). For test samples, dilute two-fold with Standard/Sample Diluent and add 100µL/well. Incubate at room temperature for 2 hours.
7. Wash plate as in Step 3.
8. Add 100µL of diluted biotin-labeled Detection Antibody solution to each well and incubate at room temperature for 1 hour.
9. Wash plate as in Step 3.
10. Add 100µL of diluted HRP-labeled Revealing Antibody solution to each well and incubate at room temperature for 30 minutes.
11. Wash plate 6 times with 1X Wash Buffer. For this final wash, soak wells in Wash Buffer for 10 seconds for each wash. This will help minimize background.
12. Add 100µL of TMB Substrate Solution to each well. Incubate at room temperature for 10-15 minutes. The Substrate Solution should turn blue in color.
13. Add 50µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells turns green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 30 minutes using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and native human G-CSF. No cross-reactivity or interference with other human proteins was observed.

Sensitivity

The minimum detectable concentration of human G-CSF is 2.7pg/mL.

Recovery

The recovery of human G-CSF spiked to levels throughout the range of the assay in serum and cell culture supernatant was evaluated by the set. At least 85% of the G-CSF was recovered from serum and 95% from cell culture supernatant.

Trouble Shooting

General technical hints:

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- 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- During incubation steps, shaking the plates may increase sensitivity.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue upon addition.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color development in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that turn green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Poor Signal

- Avoid sodium azide in wash buffers as it inhibits the enzymatic activity of HRP
- Verify that appropriate antibody pairs were used
- Inadequate reagent volumes added to wells
- Incorrect incubation times and/or temperature

Poor Standard Curve or Precision

- Improper standard handling and/or dilution
- Inadequate mixing of reagents
- Inadequate aspiration and/or washing of wells

References

1. Kamezaki, K. et al. (2005). *Stem Cells*. **23**:252-63.
2. McLemore, M. L. et al. (2001). *Immunity*. **14**:193-204.
3. Buzzeo, M.P. et al. (2007). *Exp. Hemat.* **35**:1456-65.
4. Kavgaci, H. et al. (2002). *J. Exp. Clin. Cancer Res.* **21**:475-9.
5. Krol, J. et al. (2006). *Onkol.* **29**:171-8.

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