| ELISA Kit Components | Amount | Part No. |
|---|---|---|
| Anti-Human G-CSF Microwell Plate | 8-well strips (12) | 0081 |
| Human G-CSF Standard, lyophilized | 2 vials | 0082 |
| Anti-Human G-CSF Detection Ab (100X) | 0.15 ml | 0083 |
| Streptavidin HRP Conjugate (100X) | 0.15 ml | S-HRP100 |
| Sample Diluent Concentrate (20X) | 10 ml | SD-20T |
| Wash Solution Concentrate (100X) | 10 ml | WB-100 |
| TMB Substrate | 12 ml | 80091 |
| Stop Solution | 12 ml | 80101 |
| Product Manual | 1 ea | M-0080 |

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and HRP Antibody contain bromonitrodioxane (BND: 0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and BND, if not already on file, can be requested or obtained from the ADI website.

## NOTES



*Instruction Manual No. 0080*

# Human G-CSF

**ELISA Kit Cat. No. 0080**

**For Quantitative Determination of Human Granulocyte Colony-Stimulating Factor (G-CSF) in Biological Solutions**

ALPHA DIAGNOSTIC INTERNATIONAL

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## INTENDED USE

The Human G-CSF ELISA Kit is an in vitro immunoassay for research use in the quantification of G-CSF in cultures of human cells and in appropriately qualified samples from serum, saliva, or other tissue fluids or extracts.

## RESEARCH USE OF THE TEST

Human granulocyte colony-stimulating factor (hG-CSF) is a glycoprotein cytokine or growth factor, occurring in 2 forms (174- and 180-aa) of about 19,600MW, produced by a number of different tissues, including monocytes, macrophages, fibroblasts and epithelium, to stimulate the bone marrow to produce granulocytes and stem cells. In addition, G-CSF stimulates proliferation, differentiation, survival and release into the blood of precursors and mature neutrophils.

Recombinant hG-CSF has been used as a pharmaceutical product to treat myelo-suppresive chemotherapy for cancer patients, and has especially helped advance high-dose chemotherapy regimens, by stimulating white blood cell production and allowing for neutrophil recovery. hG-CSF is also used to increase hematopoietic sstem cells in the donor prior to collection for stem cell transplantation. Recently, hG-CSF has ben conjugated to polyethyleneglycol (called pegylation) to provide an effective drug that has essentially no renal clearance, and promises to increase the dose-effectiveness of hG-CSF in circulation. The behavior of pegylated hG-CSF in this ELISA has not been investigated.

## PRINCIPLE OF THE TEST

The Human G-CSF ELISA kit is based on the binding of human G-CSF in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to biotin, which then binds to a streptavidin horseradish peroxidase (HRP) conjugate. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of hG-CSF present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of hG-CSF in samples is calculated from a standard curve of purified recombinant human G-CSF of designated concentration.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8$^{o}$C until the expiration date printed on the Kit label. Stabilities of the working solutions are indicated under Reagent Preparation.



## PERFORMANCE CHARACTERISTICS & EXPECTED RESULTS

**Specificity**
The antibodies used in this kit have been produced from animals immunized with purified recombinant human G-CSF, and affinity purified using an immunosorbent of purified recombinant human G-CSF.

The following antigens exhibited less than 1% crossreactivity when tested at 50ng/ml: human IL-6, CT-1, CNTF, GM-CSF, IL-11, M-CSF and OSM; mouse G-CSF and IL-6; rat CNTF and IL-6.

**Culture Medium**
Linearity of Dilution and Recovery
hG-CSF was spiked into Sample Diluent with 10% Neonatal Bovine Serum at 4 levels, 200-1600pg/ml. The mean recovery ranged from 91 to 110%, demonstrating linear dilution and equivalent quantification across the standard range.

**Serum & Plasma**
Linearity of Dilution and Recovery
hG-CSF was spiked into eight (8) normal human sera and plasma samples and diluted to 1600 pg/ml G-CSF and 10% serum/plasma. The mean recovery ranged from 60 to 94%.

## QUALITY CONTROL

**Reagents** Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

**Sample Controls** Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Diluent only Negative Control should also be run.

**Standard Curve** The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

**Technique** Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

## CALCULATION OF RESULTS

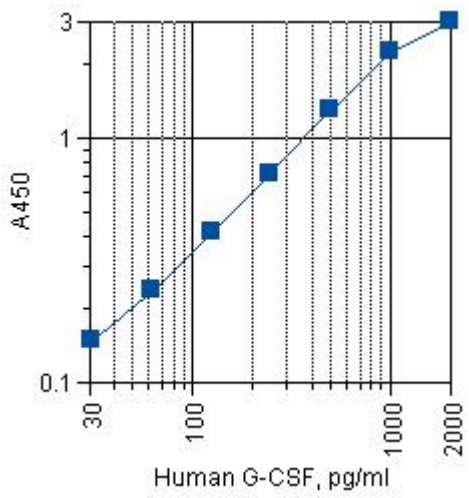
The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, hG-CSF concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the standards (y-axis) against the concentration (pg/ml) of hG-CSF (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
3. The hG-CSF concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by the dilution factor.
5. Samples producing signals higher than the 2000 pg/ml standard should be further diluted and re-assayed.

## TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

| Wells | Standards, Control & Samples | A450 nm | pg/ml |
|---|---|---|---|
| A1, A2 | **Negative Diluent Control** | 0.07 | 0 |
| B1, B2 | 31.25 pg/ml **Standard** | 0.15 | 31.25 |
| C1, C2 | 62.5 pg/ml **Standard** | 0.24 | 62.5 |
| D1, D2 | 125 pg/ml **Standard** | 0.41 | 125 |
| E1, E2 | 250 pg/ml **Standard** | 0.72 | 250 |
| F1, F2 | 500 pg/ml **Standard** | 1.31 | 500 |
| G1, G2 | 1000 pg/ml **Standard** | 2.24 | 1000 |
| H1, H2 | 2000 pg/ml **Standard** | 3.01 | 2000 |
| A3, A4 | **Sample** [Diluted 1:5] | 1.27 | 585 |
| | Calculated: 5-fold dilution x 585 pg/ml = **2.92** ng/ml in sample | | |






## KIT CONTENTS

**To Be Reconstituted:** Store as indicated.

| Component | Instructions for Use |
|---|---|
| **Human G-CSF Standard** Part No. 0082 | Two (2) vials, each containing recombinant human G-CSF, lyophilized in buffer with protein and BND as stabilizers. Keep lyophilized vials refrigerated until used or kit lot expires. |

Reconstitute 1 vial with 1.0 ml **Working Sample Diluent** to provide a 2000 pg/ml Top Standard, sufficient for two entire curves. Prepare 2-fold dilutions, as follows:

| **Standard** | **+ Diluent =** | **Final Concn** |
|---|---|---|
| Reconstituted Standard | None | 2000 pg/ml |
| 250 ul of 2000 pg/ml | 250ul | 1000 pg/ml |
| 250 ul of 1000 pg/ml | 250ul | 500 pg/ml |
| 250 ul of 500 pg/ml | 250ul | 250 pg/ml |
| 250 ul of 250 pg/ml | 250ul | 125 pg/ml |
| 250 ul of 125 pg/ml | 250ul | 62.5 pg/ml |
| 250 ul of 62.5 pg/ml | 250ul | 31.25 pg/ml |

Use within 2 weeks of preparation, if stored @ 4° C;
Or within 2 months, if stored frozen.

| | |
|---|---|
| **Sample Diluent Concentrate (20x)** Cat. No. SD-20T, 10ml | Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up. |
| **Wash Solution Concentrate (100x)** Cat. No. WB-100, 10ml | Dilute the entire volume, 10ml, to 1L with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely. |
| **Anti-Human G-CSF Detection Antibody Concentrate (100x)** Part No. 0083, 0.15ml | Biotinylated anti-human G-CSF in buffer with protein, detergents and BND as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage. |
| **Streptavidin-HRP Conjugate Concentrate (100x)** Part No. S-HRP100, 0.15ml | Peroxidase conjugated streptavidin in buffer with protein, detergents and BND as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8°C storage. |

**Ready For Use:** Store as indicated on labels.

| Component | Part No. | Amt | Contents |
|---|---|---|---|
| **Anti-Human G-CSF Microwell Strip Plate** | 0081 | 8-well strips (12) | Coated with purified anti-Human G-CSF antibodies. Return unused strips to the pouch with desiccant; re-seal and store refrigerated. |
| **TMB Substrate** | 80091 | 12 ml | Chromogenic substrate for HRP containing TMB and peroxide. |
| **Stop Solution** | 80101 | 12 ml | 1% sulfuric acid. |

**Materials Required But Not Provided:**

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples, Detection Antibody Concentrate and Streptavidin-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

## SPECIMEN COLLECTION AND HANDLING

Human serum may contain infectious material. Always wear gloves when handling serum-containing samples (standards and controls contain no human serum), and dispose of these samples and containers as biohazard waste.

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

**Samples, Standards and Controls**

Dilute **Samples** in Working Sample Diluent according to expected G-CSF levels; for serum: dilute at least 5-fold (e.g., 50 ul sample + 200 ul Diluent) for reduced nonspecific signals.

Do not dilute the **Standards**. Include Working Sample Diluent as a Negative Control to determine proper assay performance (signal should be < 0.3 OD). Internal **Controls** that represent the lab's expected results should also be included in each assay run.



## ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

**1. Set-up**

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Before sample addition, add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes.
- Aspirate or dump the liquid and pat the plate dry on a paper towel.

**2. 1st Incubation [100ul – 60 min; 4 washes]**

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer is recommended. Improper washes may lead to falsely elevated signals and poor reproducibility.

**3. 2nd Incubation [100ul – 60 min; 4 washes]**

- Add 100ul of Working Detection Antibody to each well.
- Incubate for 60 minutes.
- Wash wells 4 times as in step 2.

**4. 3rd Incubation [100ul – 30 min; 5 washes]**

- Add 100ul of Working Streptavidin-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

**5. Substrate Incubation [100ul – 15 min]**

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.
  Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, assuring the top standard does not surpass 2 OD.

**6. Stop Step [Stop: 100ul]**

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

**7. Absorbance Reading**

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.