



## G-CSF (GRANULOCYTE COLONY STIMULATING FACTOR)

The Singulex G-CSF assay enables robust investigation of the role of G-CSF in numerous disease processes such as leukemia and metastatic cancer, with notable of accuracy and precision surpassing other high-sensitivity commercial assays.

### BIOLOGY AND DISEASES

Granulocyte-Colony Stimulating Factor (G-CSF) is an endogenous hematopoietic agent activated and expressed by many types of cells, including macrophages, tumor cells, fibroblasts and endothelial cells. One of the main functions of G-CSF is to regulate formation of neutrophils during hematopoiesis, which are important for proper immune function. For example, in cancer patients, reduced hematopoiesis from chemotherapy results in low neutrophil production, which jeopardizes the immune system, leaving the body susceptible to infectious attacks.

### THERAPIES

G-CSF has been the focus of pharmaceutical development for several decades. A recombinant human G-CSF (filgrastim) was marketed under the trade name Neupogen in 1991. In 2002, Neulasta (pegfilgrastim) was released as the longer-lasting form of Neupogen. A third form of G-CSF (lenograstim), expressed in Chinese Hamster Ovary cells instead of *E. coli* as in the former two, is also available on the market. G-CSF therapy has been shown to restore hematopoiesis in cancer and leukemic patients by stimulating white blood cell production. Interestingly, G-CSF has also demonstrated effectiveness in improving cardiac function following a myocardial infarction.

### UNMET NEED

The efficacy in reducing the risk of infection following cancer treatments with G-CSF has been well established, but its direct therapeutic effect still remains unanswered. Ongoing investigations of the prognostic use of this growth factor require an assay capable of differentiating G-CSF concentrations in healthy and diseased individuals across a broad quantification range. A leukemia treatment study reported that the level of G-CSF in chronic phase patients is either low (0.9 pg/mL) or undetectable, and is much lower than that found in normal controls (22 pg/mL). Thus it is imperative to have an assay with improved sensitivity and accuracy over a spectrum of sample concentrations in order to successfully evaluate effectiveness and monitor the dosing of potential G-CSF treatments.

### SINGULEX ANSWER

The Singulex G-CSF assay, optimized for use on the Erenna System, enables scientists to measure a broad dynamic range of G-CSF in plasma. The assay has an LLoQ of 0.20 pg/mL and a reading range of 0.14-100 pg/mL, providing accurate quantification of G-CSF from only 100µL of plasma.

This assay will allow investigators to:

1. Measure the efficacy and dosing of therapeutics designed to increase levels of G-CSF, particularly in rescuing hematopoiesis in leukemic patients.
2. Accurately quantify below-normal levels of G-CSF compared to healthy subjects, such as in cases of neutropenia in chronic phase cancer patients.
3. Design more robust clinical and preclinical studies when G-CSF concentration is used as a therapeutic endpoint.
4. Establish baseline concentrations in healthy subjects and monitor changes in G-CSF levels during disease progression.

### ERENNA TECHNOLOGY ACCESS PROGRAM.

Through the Erenna Technology Access Program (ETAP), Singulex offers an interactive, results-driven solution to biomarker challenges faced by the pharmaceutical industry during product development. Singulex assists the development programs of our ETAP collaborators by developing customer-driven assays and access to a menu of fully-validated assays. Participants in ETAP gain access to the Singulex Erenna Immunoassay System, our proven expertise developing high-value immunoassays and our world-class customer support. Together with Singulex, our ETAP collaborators are expanding the utility of protein biomarkers and using them as tools to measure disease progression, drug efficacy and toxicity.

**TABLE 1:** Analytical sensitivity of the Singulex G-CSF assay.

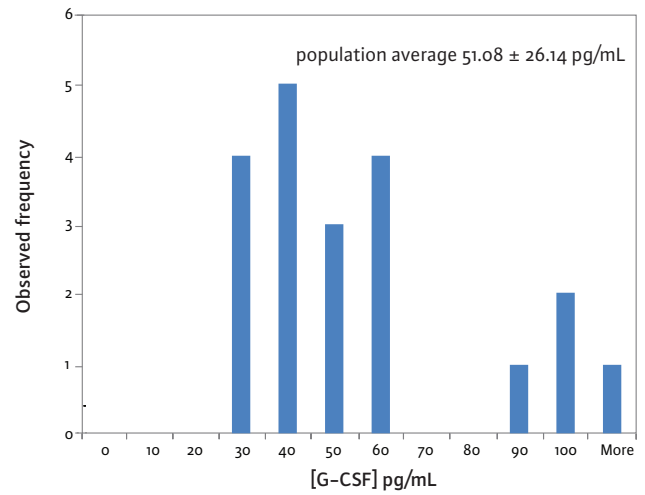
Lower Limit of Detection (LoD)	0.14 pg/mL
Lower Limit of Quantification (LLoQ)	0.20 pg/mL
Reading Range	0.14-100 pg/mL

**Erenna® System**

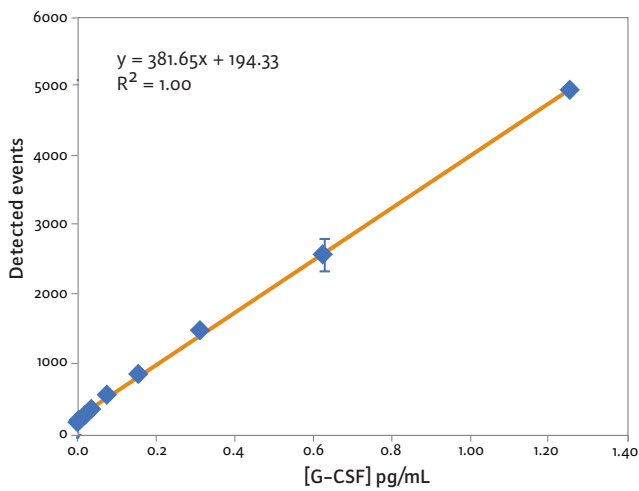


**TABLE 2:** G-CSF assay low-end standard curve data.

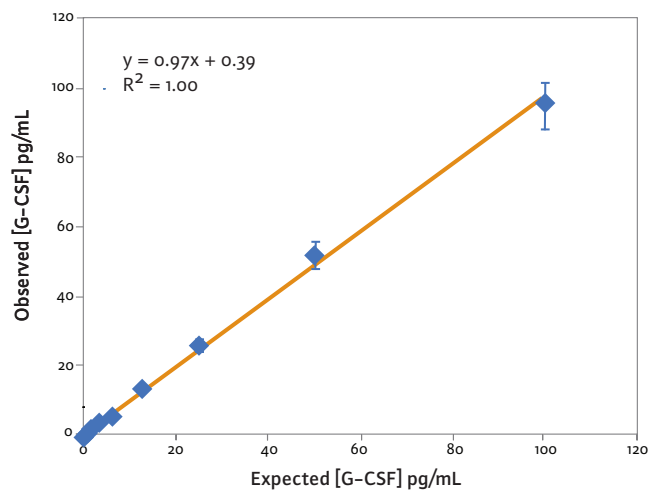
[G-CSF] pg/mL	Detected Events	Std Dev	CV
12.5	4941	63	1%
6.25	2567	247	10%
3.13	1484	39	3%
1.56	829	18	2%
0.78	540	15	3%
0.39	325	21	6%
0.20	233	11	5%
0.10	188	11	6%
0.00	145	26	18%



**FIGURE 1:** Plasma G-CSF concentration in healthy human subjects.



**FIGURE 2:** G-CSF low-end standard curve signal.



**FIGURE 3:** G-CSF assay curve fit.

These standard curves are for representational purposes only. A standard curve must be run with each assay.

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