# instaCELL G-CSF potency assay kit

## Product Information

Cat N° SF330-01

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# 1 Description

Granulocyte Colony-Stimulating Factor (G-CSF) is a naturally occurring protein produced by various cells in the body, including macrophages, monocytes, fibroblasts, and endothelial cells. It plays a crucial role in the regulation of the production, differentiation, and function of white blood cells, particularly granulocytes (neutrophils, eosinophils, and basophils), in the bone marrow [1]. Recombinant forms of G-CSF are used in medicine to treat conditions associated with neutropenia (a low neutrophil count), such as chemotherapy-induced neutropenia, myelodysplastic syndromes, and bone marrow transplantation [2].


**Figure 1:** instaCELL G-CSF potency assay kit

The instaCELL G-CSF potency assay kit is an *in vitro* test to determine the potency of G-CSF formulations for quality control. The assay utilizes the cytokine dependence of NFS-60 cells, a murine myeloid progenitor cell line, for their proliferation, which make them a valid model to determine the potency of cytokines like G-CSF in a proliferation bioassay [3]. The assay kit uses a Resazurin viability assay to analyze the potency of the G-CSF product and compares it to a kit included reference.

The instaCELL G-CSF potency assay kit is based on the WHO publication "Report on a Collaborative study for Proposed 2nd International standard for Granulocyte colony stimulating factor (G-CSF)"[4] and the parameters of the European pharmacopoeia 07/2019:2206. It includes prequalified Assay Ready NFS-60 Cells as well as buffer, Resazurin viability assay, G-CSF reference, and 96-well plate to perform the assay. Assay Ready Cells are frozen cell aliquots which can be used directly after thawing without additional cultivation, basically like a reagent.

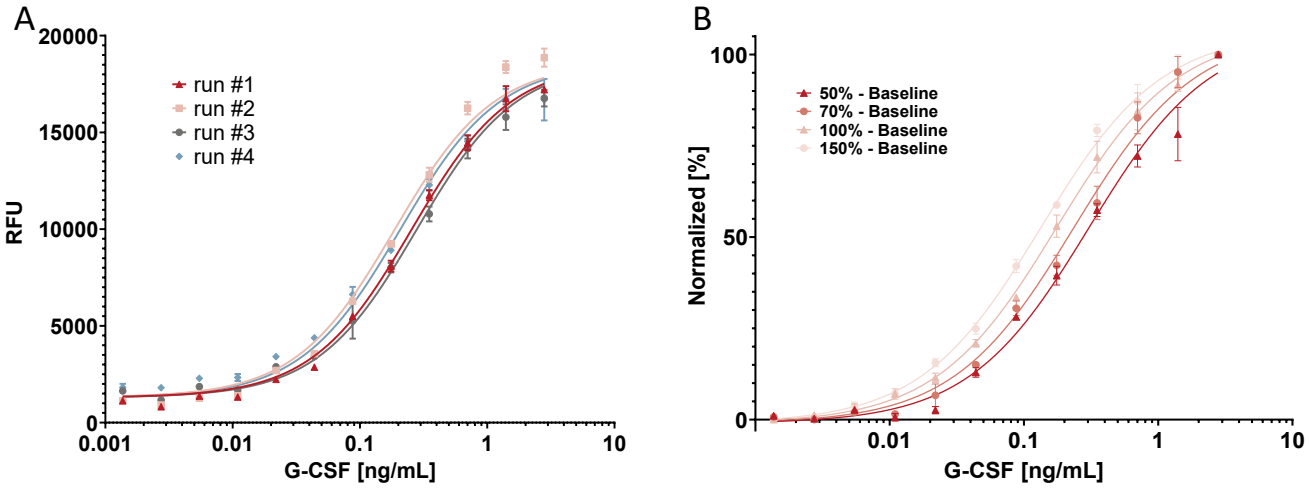


**Figure 2: Precision & Accuracy of the instaCELL G-CSF potency assay kit**

**[A]** The potency of a given G-CSF preparation can be precisely determined with NFS-60 assay ready frozen cells. In four independent experiments performed on different days using individual aliquots of assay ready NFS-60 an average EC50 of 0.23 ng/ml was determined with a CV of 9%.

**[B]** The accuracy of the G-CSF proliferation assay was tested using nominal doses of 50%, 70%, 100% and 150% measuring potencies of 51,2%, 63,7%, 100% and 137,2%, which is an average accuracy of 95%.

## 2 Cell Information

| | |
|---|---|
| Source: | Cell Line Service GmbH |
| Cell Type: | Leukemia, myeloid |
| Tissue: | Blood |
| Species: | Mouse |
| Growth: | Suspension |
| Biosafety Level: | 1 |

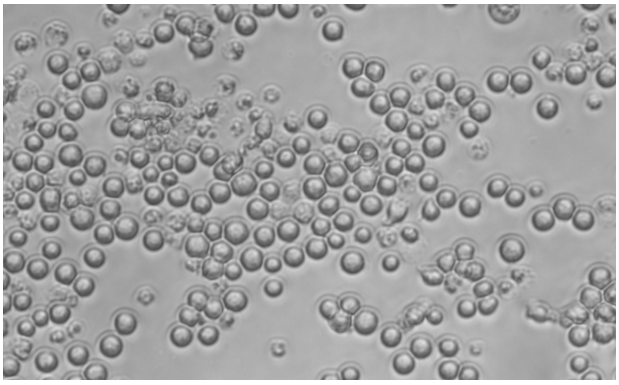

**Figure 3:** Morphology of Assay Ready NFS-60 Cells

## 3 Kit Content

| | | |
|---|---|---|
| • NFS-60 assay ready cells | 1 vial (5E+06 cells) | RE703K |
| • Recovery Buffer G | 1 bottle (10ml) | MD165-01 |
| • Assay Buffer G | 1 bottle (60ml) | MD365-06 |
| • G-CSF Reference | 1 vial (20µl) | RX519-01 |
| • Resazurin | 1 bottle (5ml) | RX718-01 |
| • 96-well plate | 1 plate | ZG02-13 |

# 4 Protocol of Use

## 4.1 Day I: Seeding of NFS-60 and addition of samples

### *4.1.1 Preparation of test sample and controls*

To avoid edge effects, the space between the wells of the assay plate can be filled with sterile liquid (e.g. PBS).

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate assay buffer to room temperature.
- Dilute the G-CSF reference **two times** 10-fold in assay buffer. First 10µl stock solution in 90µl assay buffer (dilution 1) and then 50µl of dilution 1 in 450µl assay buffer (dilution 2).
- Further dilute 68µl of dilution 2 in 1143µl assay buffer to obtain the top concentration (C1) of the G-CSF reference standard. This solution is 2x concentrated and contains 5.58 ng/ml.
- Perform a 2-fold dilution series of 12 concentrations for the G-CSF reference standard curve. Add 600µl of C1 to 600µl of assay buffer, mix well and continue this process up to C12.
- Prepare a 2x concentrated dilution series of your test sample in assay buffer, analogue to the G-CSF standard dilution series.

### *4.1.2 Thawing and seeding the cells*

- Equilibrate assay buffer to 37°C.
- Thaw one vial of Assay Ready Cells in a water bath at 37°C for 3min. Prepare 8ml of recovery buffer in a 15ml centrifugation tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer.
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant. Resuspend the cell pellet in 11ml of assay buffer.
- Dispense 75µl of the cell suspension into each well of the provided assay plate, except the wells in row A. Mix cell suspension before each pipetting step.
- Add 75µl of G-CSF reference (rows A-D) and sample (rows E-G) dilutions in triplicate to the respective wells as recommended in the plate layout (Fig 4). The plate layout has an inside-out design to avoid edge effects in relevant concentrations.
- Add 75µl of assay buffer to each well of row A and row H for blank and medium control.
- Incubate for 48h in a humidified incubator at 37°C and 5% $CO_2$.

## 4.2 Day III: Readout by Resazurin Viability Assay

- Equilibrate the resazurin solution to room temperature.
- Add 20µl of Resazurin solution to each well of the assay plate. Shake the plate for 15s and incubate it for 4h at 37°C and 5% $CO_2$.
- The fluorescence is measured at 540nm and 590nm for reference.

# 5 Plate Layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | ~~no cells C12~~ | ~~no cells C10~~ | ~~no cells C8~~ | ~~no cells C6~~ | ~~no cells C4~~ | ~~no cells C2~~ | ~~no cells C1~~ | ~~no cells C3~~ | ~~no cells C5~~ | ~~no cells C7~~ | ~~no cells C9~~ | ~~no cells C11~~ |
| B | Ref C12 | Ref C10 | Ref C8 | Ref C6 | Ref C4 | Ref C2 | Ref C1 | Ref C3 | Ref C5 | Ref C7 | Ref C9 | Ref C11 |
| C | Ref C12 | Ref C10 | Ref C8 | Ref C6 | Ref C4 | Ref C2 | Ref C1 | Ref C3 | Ref C5 | Ref C7 | Ref C9 | Ref C11 |
| D | Ref C12 | Ref C10 | Ref C8 | Ref C6 | Ref C4 | Ref C2 | Ref C1 | Ref C3 | Ref C5 | Ref C7 | Ref C9 | Ref C11 |
| E | sample C12 | sample C10 | sample C8 | sample C6 | sample C4 | sample C2 | sample C1 | sample C3 | sample C5 | sample C7 | sample C9 | sample C11 |
| F | sample C12 | sample C10 | sample C8 | sample C6 | sample C4 | sample C2 | sample C1 | sample C3 | sample C5 | sample C7 | sample C9 | sample C11 |
| G | sample C12 | sample C10 | sample C8 | sample C6 | sample C4 | sample C2 | sample C1 | sample C3 | sample C5 | sample C7 | sample C9 | sample C11 |
| H | medium control | | | | | | | | | | | |

test sample (triplicates of 12 dilutions), reference standard (triplicates of 12 dilutions), reference standard no cells
medium control

# 6 Analysis and Assay Acceptance Criteria

## 6.1 Calculation of Viability and $EC_{50}$

- Calculate the mean of the wells of row A and subtract it from each well to remove the background signal.
- Calculate the mean of the medium control and subtract it from each well for base line correction.
- Calculate the viability for concentration of the test sample and reference standard using the following equation:

$$\frac{Mean\ of\ Triplicate}{Mean\ of\ C1\ form\ reference}\ x\ 100\% = Viability\ [\%]$$

**Equation 1: Calculation of the cell viability**

- Calculate the $EC_{50}$ with a four-parameter fit model, the X axis contains the concentration of the G-CSF and the Y axis the calculated viability.

## 6.2 Acceptance Criteria

- The $EC_{50}$ value for the G-CSF reference standard should be between 0.1 ng/ml and 0.4 ng/ml.
- The potency for the test sample must not be less than 80% and no more than 125% related to the G-CSF reference standard.
- The confidence limit (P = 0.95) must not be below 74% and not above 136%.

For results outside these limits, the assay must be repeated.

## 7 Stability & Storage

Performance of the kit is guaranteed within the specifications as defined in the certificate of analysis only before the expiration date as indicated on the packaging and only if stored and handled according to the instruction of this datasheet.

- Store Assay Ready Cells below -140°C (e.g., in vapor phase of liquid nitrogen).
- Store all other reagents and media as indicated on the label.

## 8 Literature & Related Documents

[1] Bath PM, Sprigg N. Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. Cochrane Database Syst Rev. 2007 Apr 18;(2):CD005207. doi: 10.1002/14651858.CD005207.pub3. Update in: Cochrane Database Syst Rev. 2013;6:CD005207. PMID: 17443577.

[2] Hollingshead LM, Goa KL. Recombinant granulocyte colony-stimulating factor (rG-CSF). A review of its pharmacological properties and prospective role in neutropenic conditions. Drugs. 1991 Aug;42(2):300-30. doi: 10.2165/00003495-199142020-00009. PMID: 1717226.

[3] Hara K, Suda T, Suda J, et al. Bipotential murine hemopoietic cell line (NFS-60) that is responsive to IL-3, GM-CSF, G-CSF, and erythropoietin. Experimental Hematology. 1988 May;16(4):256-261. PMID: 2452092.

[4] Bird, C. et al., Report on a collaborative study for proposed 2nd international standard for granulocyte colony stimulating factor (G-CSF). / by Meenu Wadhwa, Chris Bird, Michelle Hamill, Alan B Heath, Robin Thorpe. Switzerland.

## 9 Support

https://www.accellerate.me/support/contact.html

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## 10 Disclaimer

The product is sold under the terms of a Limited Use Label License attached to the product. By breaking the seal of the product package, the user explicitly agrees to the license terms. Assay Ready Cells are for immediate assay use only. The user shall not propagate, passage, or refreeze the cells.

This product is intended for research use only. Do not use for diagnostic or therapeutic purposes.