Quantification of functional G-CSF using *iLite*® G-CSF Assay Ready Cells

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

This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.

Background

Granulocyte-colony stimulating factor (G-CSF or GCSF) is a glycoprotein that stimulates the production of granulocytes and stem cells in the bone marrow, and their subsequent release into the bloodstream. Bone marrow derived precursor cells expressing the G-CSF receptor on the cell surface respond with initiation of proliferation and differentiation after binding of G-CSF to its receptor. Pharmaceutical G-CSF analogs are used as supporting treatment to stimulate production of granulocytes (white blood cells) in patients with neutropenia due to chemotherapy, to accelerate recovery and reduce mortality (1,2).

Principle of the assay

The *iLite*® G-CSF Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of a G-CSF responsive promoter. Binding of G-CSF to the G-CSF receptor (GSF3R) results in activation of the G-CSF regulated Firefly luciferase reporter gene construct. *iLite*® G-CSF Assay Ready Cells also contain the Renilla Luciferase (RL) reporter gene, under the control of a constitutive promoter. The constitutive expression of RL allows normalization of G-CSF induced FL activity, and renders assay results independent of variations in cell number or serum matrix effects. The luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. The Firefly luciferase signal is proportional to the concentration of functional G-CSF in a sample (Fig.1).

Specimen collection

The *iLite*® G-CSF Assay Ready Cells can be used for measuring concentration of G-CSF in test samples including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® G-CSF Assay Ready Cells	Svar Life Science	BM4055
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
G-CSF or analogues	R&D Systems	214-CS-005/CF
Firefly/Renilla luciferase substrate	Promega	E2920, Dual-Glo Luciferase Assay System
Plate; White walled micro well plate suitable for luminescence	PerkinElmer	6005680
Microplate Luminometer with appropriate reading software – no filter on luminometer	Contact Svar Life Science for list of recommended suppliers	NA
Incubator, 37 °C with 5% CO_2	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA

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Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of calibrators (G-CSF)

Recombinant G-CSF from R&D Systems has successfully been used to stimulate the *iLite*® G-CSF Assay Ready Cells. The below table shows the dilutions of G-CSF, used for QC release of the *iLite*® G-CSF Assay Ready Cells.

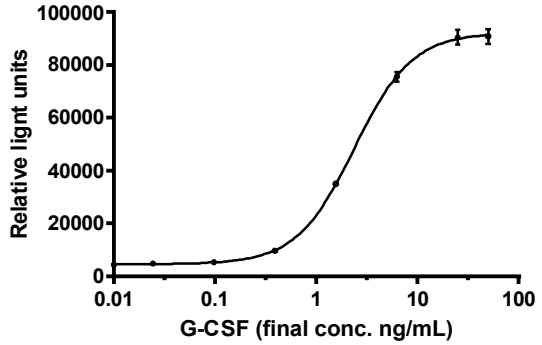

Figure 1. Example of G-CSF calibration curve.

Calibrator	G-CSF Suggested calibrator solution conc. (ng/ml)
A	100
B	50
C	13
D	3.1
E	0.78
F	0.20
G	0.049
H	0

Table 1. Suggested calibrator **solution concentrations** for G-CSF.

Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Dilute calibrators, controls and samples to fall within the expected **in assay values** of 0-50 ng/mL.
3. Add 40 µL calibrators, controls and samples in duplicate to assigned wells (final concentration will be half of solution concentration).
4. Thaw the vial of *iLite*® G-CSF Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette in order to ensure a homogeneous distribution of cells.
5. Dilute 250 µL cell suspension with 5.75 mL Diluent.
6. Add 40 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO_2.

Adding substrate solutions

8. Equilibrate the plate and the substrate solution to room temperature.
9. Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.
10. If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.

Precautions

- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the supplier's/manufacturer's instructions or product specifications accompanying the individual material and instruments.
- Dispose of all sample specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as though potentially infectious.
- Residues of chemicals and preparations are generally considered as biohazardous waste and should be inactivated prior to disposal by autoclaving or using bleach. All such materials should be disposed of in accordance with established safety procedures.

Proprietary Information

In accepting delivery of *iLite*® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third-party recipient, and only to use them directly in assays. *iLite*® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered *iLite*® Assay Ready Cells is an infringement of these patents.

QUICK GUIDE

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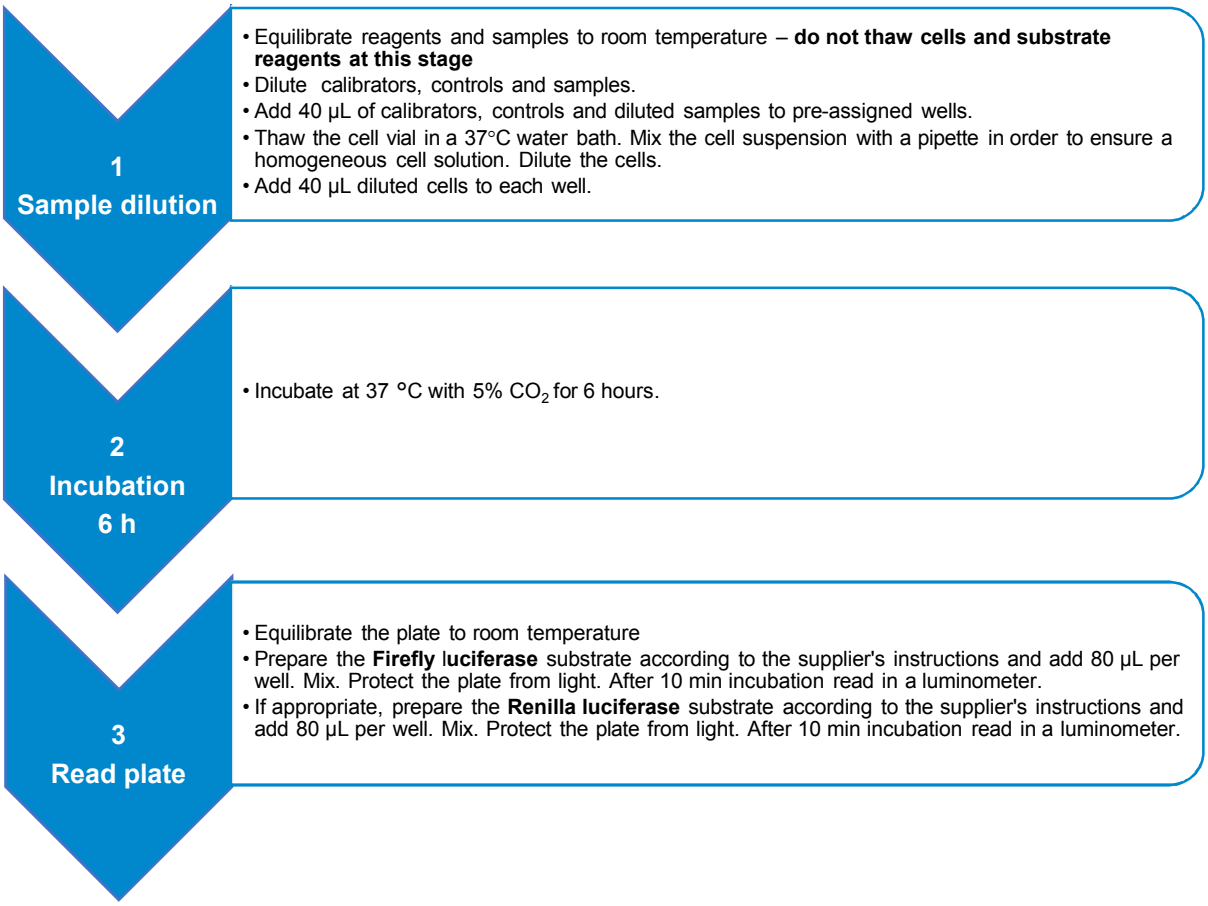

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com

References

1. Deotare U, Al-Dawsari G, Couban S, Lipton JH (September 2015). "G-CSF-primed bone marrow as a source of stem cells for allografting: revisiting the concept". Bone Marrow Transplantation. 50 (9): 1150–6.
2. Tay J, Levesque JP, Winkler IG (December 2016). "Cellular players of hematopoietic stem cell mobilization in the bone marrow niche". International Journal of Hematology. 105: 129–140.

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