Quantification of G-CSF inhibitor 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).
The assessment of factors influencing the G-CSF – G-CSF-receptor interaction such as neutralizing antibodies or other antagonists is of high importance. The iLite® platform offers a cell-based assay that enables the study of G-CSF and its receptor.

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 Firefly luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. The Firefly luciferase signal is proportional to the functional activity of G-CSF in the sample. In the presence of inhibitory activity against G-CSF, the amount of free G-CSF is reduced, resulting in a decreased stimulation of Firefly luciferase production.

Thus, the Firefly luciferase signal is inversely proportional to the amount of inhibitory activity against G-CSF in a sample. The *iLite*® G-CSF Assay Ready Cells can therefore be utilized as a highly sensitive assay for quantification of G-CSF inhibitor activity in test samples, including human serum.

Svar Life Science AB
Lundavägen 151
SE-212 24 Malmö
Sweden

P.O. Box 50117
SE-202 11 Malmö
Sweden

info@svarlifescience.com
+46 40 53 76 00

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)
Mouse anti-human G-CSF monoclonal antibody	R&D Systems	MAB214
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
Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of G-CSF inhibitor

G-CSF inhibitor from R&D Systems has successfully been used to neutralize G-CSF and inhibit the G-CSF regulated Firefly luciferase expression in *iLite*® G-CSF Assay Ready Cells (refer to the table and graph below).

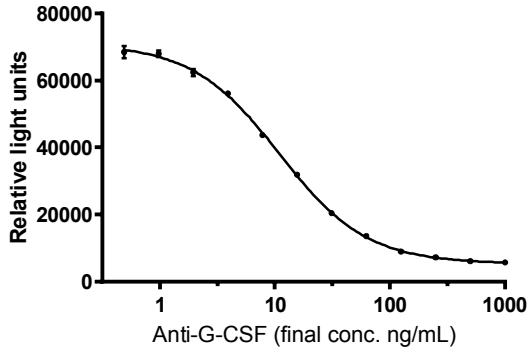


Figure 1. Example of G-CSF inhibitory curve

Final 5 ng/mL G-CSF	MAb anti-G-CSF Suggested calibrator solution concentrations, ng/mL
A	4 000
B	2 000
C	1 000
D	500
E	250
F	125
G	63
H	31
I	16
J	7.8
K	3.9
L	0

Table 1. Suggested calibrator **solution concentrations** for anti-G-CSF MAB214

Svar Life Science AB
Lundavägen 151
SE-212 24 Malmö
Sweden
P.O. Box 50117
SE-202 11 Malmö
Sweden
info@svarlifescience.com
+46 40 53 76 00

Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicate.
2. Perform a serial dilution of the reference G-CSF inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference G-CSF inhibitor dilutions, controls and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Add 20 µL of 20 ng/ml G-CSF to all wells (final concentration will be 5 ng/mL G-CSF).
5. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO_2.
6. 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.
7. Dilute 250 µL cell suspension with 5.75 mL Diluent.
8. Add 40 µL diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO_2.

Adding substrate solutions

10. Equilibrate the plate and the substrate solution to room temperature.
11. Prepare the **Firefly luciferase** substrate according to the suppliers 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.
12. If appropriate, prepare the **Renilla luciferase** substrate according to the suppliers 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

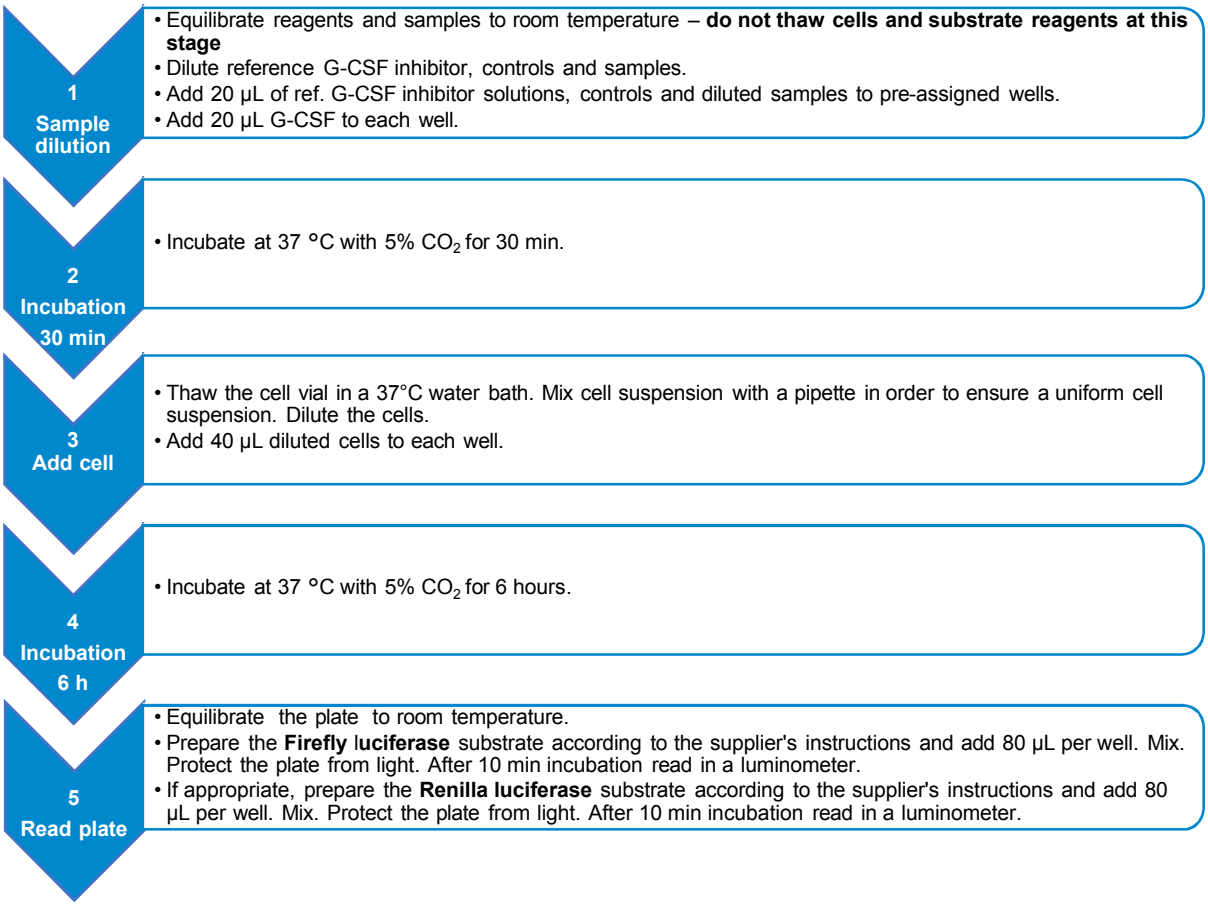
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according 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.

Propriety 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

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



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.

Svar Life Science AB
Lundavägen 151
SE-212 24 Malmö
Sweden
P.O. Box 50117
SE-202 11 Malmö
Sweden
info@svarlifescience.com
+46 40 53 76 00