Determination of GM-CSF neutralizing activity using *iLite*® GM-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-macrophage colony-stimulating factor (GM-CSF) is a cytokine stimulating the production of bone marrow derived granulocytes and monocytes precursor cells (1). GM-CSF serves as an important key in both humoral and cell mediated immunity. Recombinant GM-CSF is used in different therapeutic applications for example, to accelerate leukocyte recovery after bone marrow transplantation, to replenish leukocytes after chemotherapy and for treatment of fungal infections. The immunostimulatory effects of GM-CSF have also been used to engineer oncolytic viruses. Genes encoding for GM-CSF were introduced into the virus genome, thus enhancing the immune response to eliminate tumor cells. In addition, the discovery of a pro-inflammatory role of GM-CSF in autoimmune disease has led to development of several GM-CSF inhibitor drugs (2, 3).
Prolonged therapies with GM-CSF or GM-CSF inhibitors may lead to development of neutralizing antibodies (NAbs), which can counteract the activity of the pharmaceutical GM-CSF or GM-CSF inhibitor. The *iLite*™ GM-CSF Assay Ready Cells can be used for measurements of GM-CSF inhibitor activity and presence of neutralizing antibodies towards GM-CSF or GM-CSF inhibitors.

Principle of the assay

The *iLite*® GM-CSF Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of a GM-CSF responsive promoter. Binding of GM-CSF to the GM-CSF receptor (GM-CSFR) results in activation of the GM-CSF regulated Firefly luciferase reporter gene construct. 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 concentration of functional GM-CSF in the sample. In the presence of inhibitory activity against GM-CSF, whether as an inhibitory drug or as NAbs against GM-CSF, the amount of free GM-CSF is reduced, resulting in a decreased stimulation of Firefly luciferase expression. The Firefly luciferase signal is thus inversely proportional to the amount of inhibitory activity against GM-CSF in a sample. Hence, the *iLite*® GM-CSF Assay Ready Cells can be utilized as a highly sensitive assay for quantification of GM-CSF inhibitor activity or neutralizing antibodies against GM-CSF 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® GM-CSF Assay Ready Cells	Svar Life Science	BM4050
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Human GM-CSF, premium grade	Miltenyi Biotec	130-093-864
Human GM-CSF Antibody	R&D Systems	MAB215
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 GM-CSF inhibitor

Anti-GM-CSF antibody from Miltenyi Biotec has successfully been used to neutralize GM-CSF and inhibit the GM-CSF regulated Firefly luciferase expression in *iLite*® GM-CSF Assay Ready Cells (refer to the table and graph below).

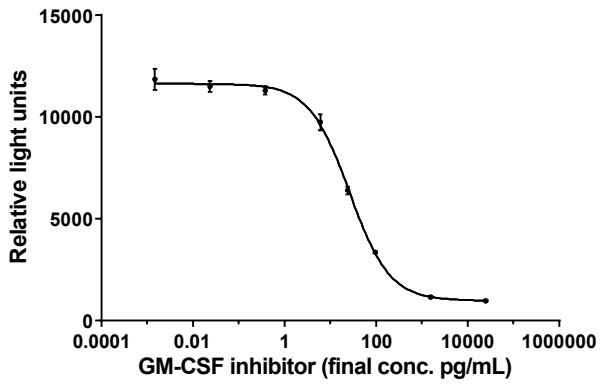

Figure 1. Example of GM-CSF inhibitory curve

Final GM-CSF 40 pg/mL	MAb anti-GM-CSF Suggested calibrator solution concentrations, ng/mL
A	100 000
B	6 250
C	391
D	98
E	24
F	1.5
G	0.095
H	0.0060

Table 1. Suggested calibrator **solution concentrations** for anti-GM-CSF MAB215

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at minimum in duplicates.
2. Perform a serial dilution of the reference anti-GM-CSF antibody. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference anti-GM-CSF antibody dilutions, controls and samples to assigned wells (final concentration will be a quarter of the solution concentration).
4. Add 20 µL of 160 pg/mL GM-CSF to all wells (final concentration will be 40 pg/mL GM-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*® GM-CSF Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully several times to ensure a homogeneous distribution of cells.
7. Dilute 250 µL cells 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 5 hours at 37°C with 5% CO_2.

Adding substrate solutions

10. Equilibrate the plate and the substrate solutions to room temperature.
11. 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.
12. 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 suppliers'/manufacturers' 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

Quantification of GM-CSF neutralizing activity using *iLite*® GM-CSF Assay Ready Cells

1 Sample dilution

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Serial dilute reference anti-GM-CSF antibody
- Add 20 µL of ref. anti-GM-CSF antibody solutions, controls and samples to pre-assigned wells
- Add 20 µL of GM-CSF to each well

2 Incubation 30 minutes

- Incubate at 37 °C with 5% CO_2 for 30 minutes

3 Add cells

- Thaw the vial of cells in a 37°C water bath. Mix cell suspension with a pipette in order to ensure a uniform cell suspension. Dilute the cells
- Add 40 µL diluted cells to each well

4 Incubation 5 hours

- Incubate at 37°C with 5% CO_2 for 5 hours

5 Read plate

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

Troubleshooting and FAQ

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

References

1. **Burgess AW, Camakaris J, Metcalf D.** *Purification and properties of colony-stimulating factor from mouse lung-conditioned medium.* Journal of Biological Chemistry 252(6):1998-2003 (1977).
2. **Hamilton, JA.** *Colony-stimulating factors in inflammation and autoimmunity.* Nature Reviews Immunology 8(7):533-44 (2008).
3. **Kim JH, Oh JY, Park BH, Lee DE, Kim JS, Park HE, Roh MS, Je JE, Yoon JH, Thorne SH, Kirn D, Hwang TH.** *Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF*. Molecular Therapy 14(3):361-70 (2006).

Svar Life Science AB

Lundavägen 151
SE-212 24 Malmö
Sweden

P.O. Box 50117
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+46 40 53 76 00