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

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

Background

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine which stimulates the production of granulocytes and monocytes from bone marrow precursors (1). As such, it serves as an important key in both humoral and cell mediated immunity. Recombinant GM-CSF has several therapeutic uses; in order 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 in oncolytic viruses modified to include genes coding for GM-CSF, thus enhancing recruitment of the immune response to 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).

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 (Fig.1).

Specimen collection

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

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite™ GM-CSF Assay Ready Cells	Euro Diagnostica	BM4050
Diluent (RPMI + 9% heat inactivated FBS + 1% Penicillin Streptomycin)	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
GM-CSF or analogues	Miltenyi Biotec	130-093
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 Euro Diagnostica 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

Preparation of calibrators (GM-CSF)

GM-CSF from Miltenyi Biotec has successfully been used to stimulate the *iLite*™ GM-CSF Assay Ready Cells. The below table shows the dilutions of GM-CSF, used for QC release of the *iLite*™ GM-CSF Assay Ready Cells.

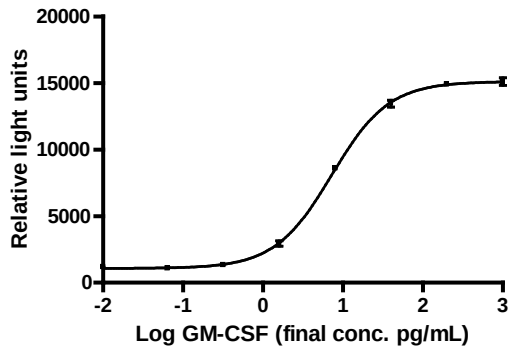


Figure 1. Example of GM-CSF calibration curve

Calibrator	GM-CSF Calibrator conc. (pg/mL)
A	1000
B	200
C	40
D	8.0
E	1.6
F	0.32
G	0.064
H	0

Table 1. Suggested calibrator concentrations for GM-CSF (final conc)

Protocol

Incubation

1. Design a plate layout.
2. Dilute calibrators, controls and samples to fall within the expected assay values of 0-1000 pg/mL.
3. Add 40 µL calibrators, controls and samples in duplicate to assigned wells.

4. 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 ten times with a pipette in order to ensure a uniform solution of cells.
5. Dilute 2 times 125 µL cells 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 5 hours at 37 °C with 5% CO_2.

Adding substrate solutions

8. Equilibrate the plate and the substrate solutions to room temperature.

9. Prepare the **Firefly luciferase** substrate according to the suppliers instructions and add 80 µL per well. Mix and protect the plate from light. Read in a luminometer after 10 minutes incubation at room temperature.

10. 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. Read in a luminometer after 20 minutes incubation at room temperature.

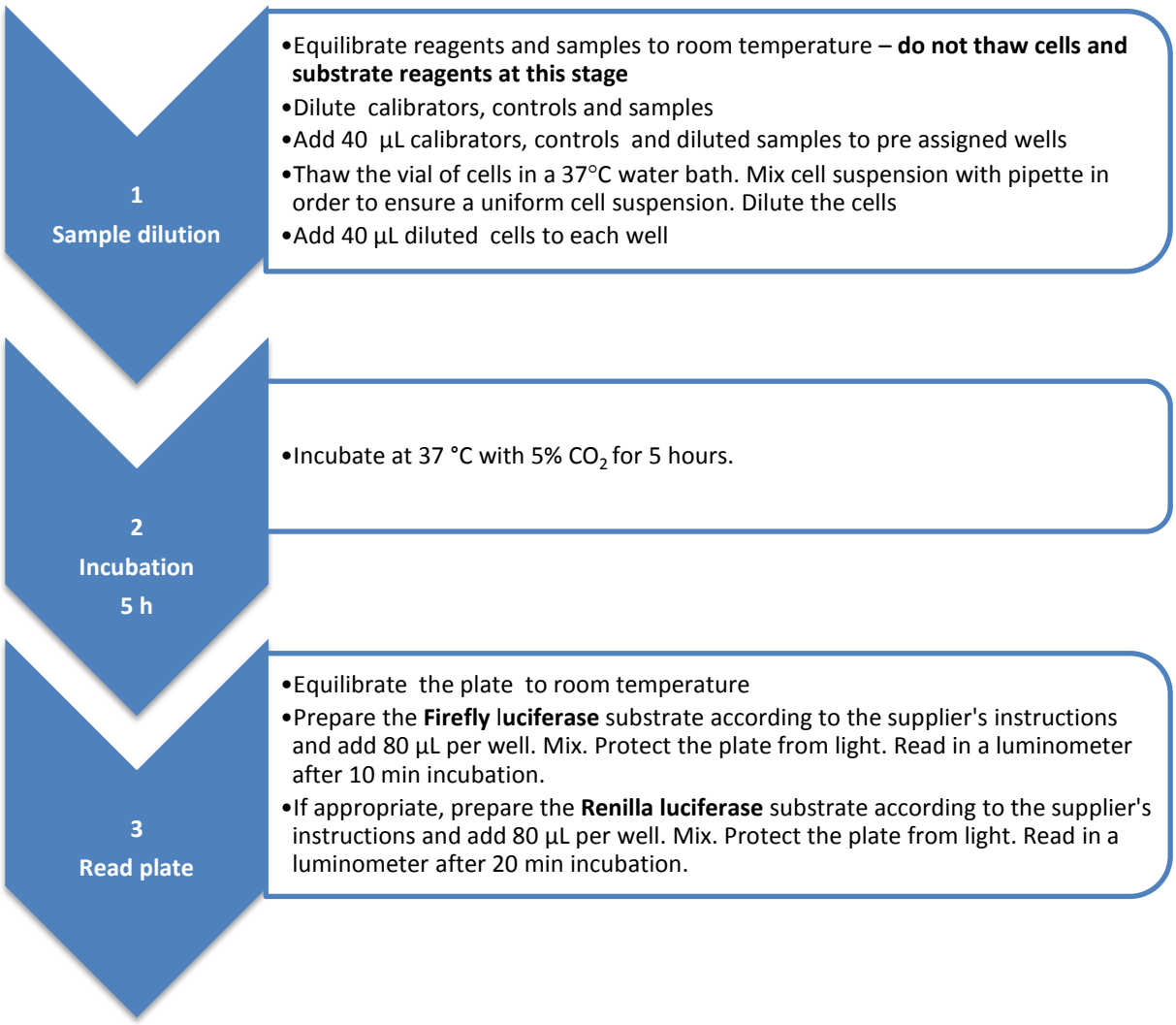
Precautions

- 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. Biomonitor *iLite™* cell-based products are covered by patents which are the property of Euro Diagnostica AB and any attempt to reproduce the delivered *iLite™* Assay Ready Cells is an infringement of these patents.

Quick Guide – Quantification of GM-CSF activity using *iLite™* GM-CSF Assay Ready Cells



Troubleshooting and FAQ

Please consult Euro Diagnostica's website www.eurodiagnostica.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).

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