instaCELL GM-CSF potency assay kit

Product Information

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Content

1 Description

Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) is primarily produced by various immune cells, including T cells, B cells, macrophages, and endothelial cells, in response to infection, inflammation, or immune system activation [1]. Recombinant forms of GM-CSF are used for white blood cell recovery following autologous bone marrow transplantation or chemotherapy and for the treatment of fungal infections [2]. GM-CSF Potency assays are essential to ensure the quality and consistency of GM-CSF products and to confirm that they can stimulate the desired biological responses in target cells.

Figure 1: instaCELL GM-CSF potency assay kit

The instaCELL GM-CSF potency assay kit is an *in vitro* test to determine the potency of GM-CSF formulations for quality control. The assay utilizes the cytokine dependence of TF-1 cells, a human erythroblast cell line, for their proliferation, which make them a valid model to determine the potency of cytokines like GM-CSF in a proliferation bioassay [3]. The assay kit uses a Resazurin viability assay to analyze the potency of the GM-CSF product and compares it to a kit included reference.

The instaCELL GM-CSF potency assay kit is based on the WHO publication " The international standard for granulocyte-macrophage colony stimulating factor (GM-CSF) Evaluation in an international collaborative study "[4] and the parameters of the European pharmacopoeia 01/2008:1641. It includes prequalified Assay Ready TF-1 Cells as well as buffer, Resazurin viability assay, GM-CSF reference, and a 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.

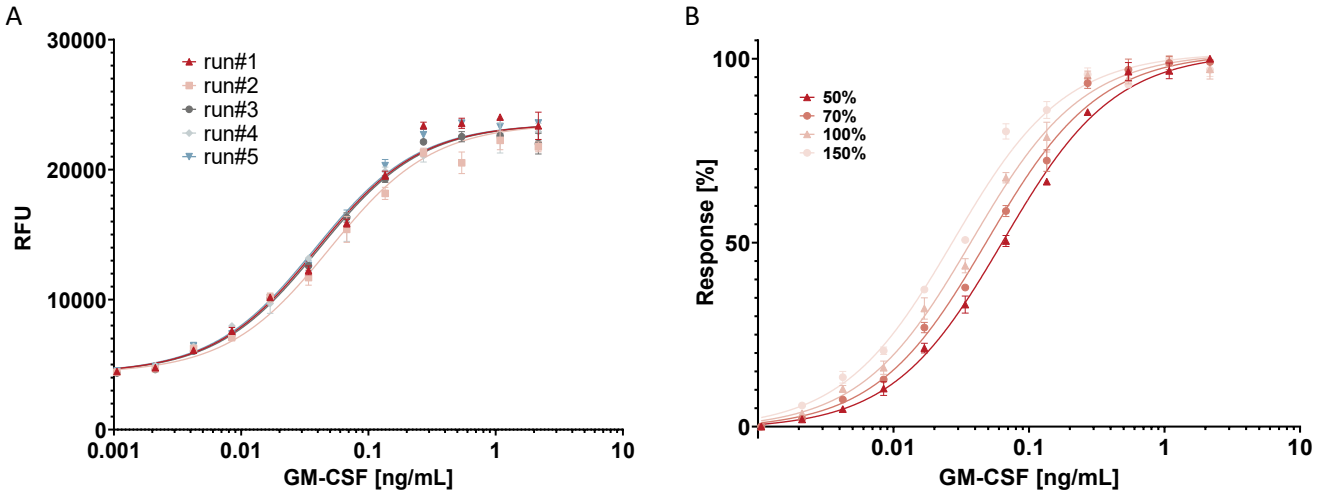

Figure 2: Precision & Accuracy of the instaCELL GM-CSF potency assay kit

[A] The potency of a given GM-CSF preparation (see methods) can be precisely determined with TF-1 assay ready frozen cells. In four independent experiments performed on different days using individual aliquots of assay ready NFS-60 an average EC_{50} of 0.0385 ng/ml was determined with a CV of 12%.

[B] The accuracy of the GM-CSF proliferation assay was tested using nominal doses of 50%, 70%, 100% and 150% measuring potencies of 58.5%, 77.0%, 100% and 136.7%, which is an average accuracy of 92%.

2 Cell Information

Source:	Cell Line Service GmbH
Cell Type:	Erythroleukemia
Tissue:	Bone marrow
Species:	human
Gender:	male
Growth:	Suspension
Biosafety Level:	1

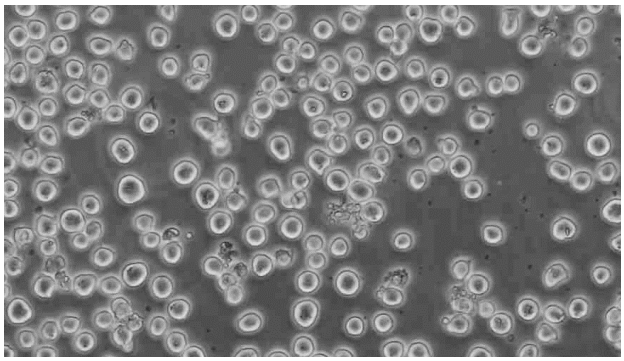

Figure 3: Morphology of Assay Ready TF-1 Cells

3 Kit Content

• TF-1 assay ready cells	1 vial (2.5E+06 cells)	RE509K
• Recovery Buffer M	1 bottle (10ml)	MD164-01
• Assay Buffer M	1 bottle (60ml)	MD364-06
• GM-CSF Reference	1 vial (20µl)	RX518-01
• Resazurin	1 bottle (5ml)	RX718-01
• 96-well plate	1 plate	ZG02-13

4 Protocol of Use

4.1 Day I: Seeding of TF-1 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 GM-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 52µl of dilution 2 in 1148µl assay buffer to obtain the top concentration (C1) of the GM-CSF reference standard. This solution is 2x concentrated and contains 4.33 ng/ml.
- Perform a 2-fold dilution series of 12 concentrations for the GM-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 GM-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 12.5ml 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 GM-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 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											

Figure 4 Recommended plate layout for the instaCELL GM-CSF potency assay kit

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

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:

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

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 GM-CSF and the Y axis the calculated viability.

6.2 Acceptance Criteria

- The EC_{50} value for the GM-CSF reference standard should be between 20 pg/ml and 60 pg/ml.
- The potency for the test sample must not be less than 80% and no more than 125% related to the GM-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] Root RK, Dale DC. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: comparisons and potential for use in the treatment of infections in nonneutropenic patients. J Infect Dis. 1999 Mar;179 Suppl 2:S342-52. doi: 10.1086/513857. PMID: 10081506.

[2] Francisco-Cruz A, Aguilar-Santelises M, Ramos-Espinosa O, Mata-Espinosa D, Marquina-Castillo B, Barrios-Payan J, Hernandez-Pando R. Granulocyte-macrophage colony-stimulating factor: not just another haematopoietic growth factor. Med Oncol. 2014 Jan;31(1):774. doi: 10.1007/s12032-013-0774-6. Epub 2013 Nov 22. PMID: 24264600.

[3] Kitamura T, Tange T, Terasawa T, Chiba S, Kuwaki T, Miyagawa K, Piao YF, Miyazono K, Urabe A, Takaku F. Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. J Cell Physiol. 1989 Aug;140(2):323-34. doi: 10.1002/jcp.1041400219. PMID: 2663885.

[4] Mire-Sluis AR, Das RG, Thorpe R. The international standard for granulocyte-macrophage colony stimulating factor (GM-CSF). Evaluation in an international collaborative study. Participants of the Collaborative Study. J Immunol Methods. 1995 Feb 13;179(1):127-35. doi: 10.1016/0022-1759(94)00273-y. PMID: 7868919.

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.