

Product Sheet

GM-CSF Reporter Cell Line

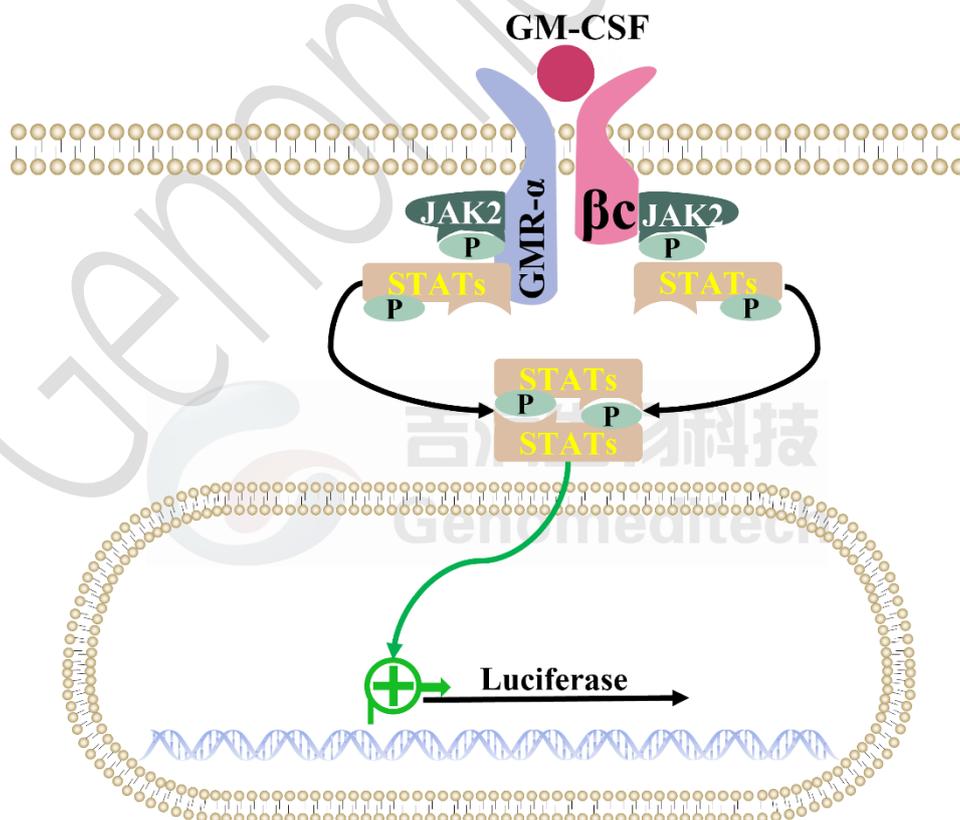
Catalog number: GM-C15752

Version 3.3.1.250103

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is a key cytokine produced by T cells, macrophages, fibroblasts, and endothelial cells. It is vital for hematopoiesis and immune responses, stimulating the proliferation and differentiation of hematopoietic stem cells in the bone marrow to produce granulocytes (like neutrophils) and macrophages.

GM-CSF signaling is activated when it binds to its receptor, which consists of two subunits: the alpha (GM-CSF-R α) and beta (β c) subunits. This binding activates JAK (Janus kinase), leading to the phosphorylation of STAT (signal transducer and activator of transcription) proteins, particularly STAT5. The GM-CSF pathway can also activate other pathways, such as PI3K/Akt and MAPK, enhancing cell survival and function.

GM-CSF Reporter Cell Line is a clonal stable cell line constructed using lentiviral technology, endogenously expresses GMR- α and β chain, along with signal-dependent expression of a luciferase reporter gene. When GM-CSF binds to its receptor, it activates downstream signaling pathways, leading to the expression of luciferase. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to GM-CSF.



Specifications

Quantity	3E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt

Recovery Medium	RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF
Growth medium	RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF+3 µg/mL Blastincidin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
Growth Conditions	37°C, 5% CO ₂

Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	gibco/C11875500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Recombinant Human GM-CSF	Novoprotein/C003
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040503

Figures

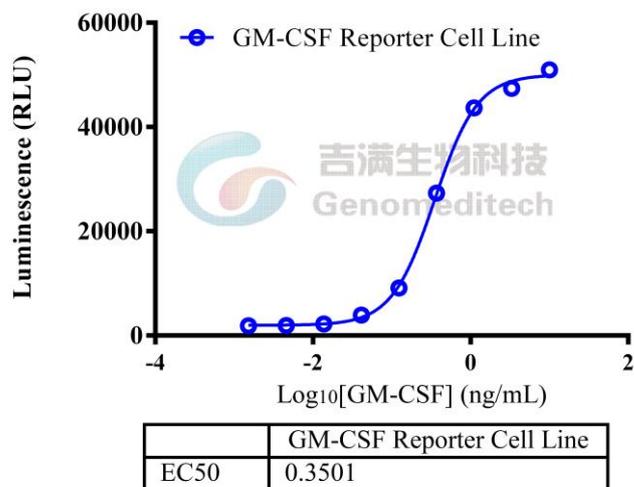


Figure 1 | Response to Recombinant Human GM-CSF. The GM-CSF Reporter Cell Line (Cat. GM-C15752) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human GM-CSF (Novoprotein/C003) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 24 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [27.5]. Data are shown by drug mass concentration.

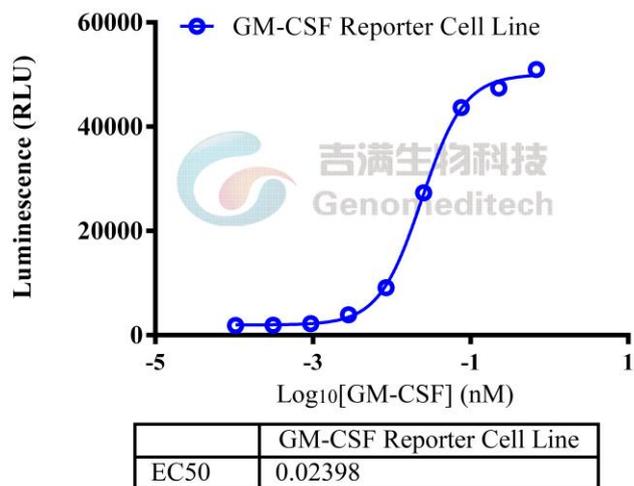


Figure 2 | Response to Recombinant Human GM-CSF. The GM-CSF Reporter Cell Line (Cat. GM-C15752) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human GM-CSF (Novoprotein/C003) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 24 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [27.5]. Data are shown by drug molar concentration.

Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability.

- a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately $176 \times g$ for 5 minutes. Discard supernatant.
- d) Resuspend the cell pellet using the recommended complete medium and adjust the viable cell density to $4\text{-}6 \times 10^5$ cells/mL. Then dispense the suspension into an appropriate culture flask and initially place the flask in an upright position after thawing.
- e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- a) Centrifuge at $176 \times g$ for 3 minutes to collect cells.
- b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 3×10^6 cells/mL.
- c) Aliquot 1 mL into each vial.
- d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF+3 $\mu\text{g}/\text{mL}$ Blastincidin

Approximately 48 - 72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics.

- a) This cell is a human erythroid leukemia cell, lymphoblast, growing in suspension.
- b) In the suspension, they appear as large, single, round cells. Cells shed a large accumulation of cytoplasmic granules in the culture, which should not be confused with bacteria!
- c) When the cell density reaches $1\text{-}1.2 \times 10^6$ cells/mL, perform a 1:2 to 1:3 split, ensuring subculturing every other day. It is essential to perform a full-volume centrifugation and medium replacement during passaging. Do not let the density exceed 1.2×10^6 cells/mL. It is recommended to use T-25 flasks for subculturing, and you can control the cell density for subculturing by counting.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 4×10^5 and 6×10^5 viable cells/mL.

Medium Renewal: Every other day

Notes

- a) To minimize the presence of cytoplasmic granules, it is essential to passage the cells every other day when the cell density reaches 1-1.2E6 cells/mL. During passaging, perform a complete centrifugation and replace the culture medium to ensure appropriate cell density and cytokine concentration. Failure to do so may promote the growth of factor-independent subclones.

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