HL-60 DeltaF508-CF Human Promyelocytic Cell Line

Cancer Cell Line

Cat. # SCC251

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

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Data Sheet

Background

Cystic fibrosis (CF) is caused by mutations in the *CFTR* gene, which encodes a chloride and bicarbonate ion channel.[1] Among the approximately 150 known disease-causing mutations in *CFTR*, deletion of the phenylalanine-508 codon (ΔF508) is the most common and results in protein destabilization, misfolding and ultimately degradation.[2] Although the most pronounced lung disease symptoms of CF, including chronic bacterial infection and purulent airway obstruction, are highly associated with neutrophils, the lack of cellular models for CF neutrophils has hindered progress in understanding the significance of these cells for CF pathogenesis.[3]

The HL-60 DeltaF508-CF human promyelocytic cell line is a derivative of the HL-60 promyelocytic leukemia cell line that has been edited via CRISPR/Cas9 technology for homozygous deletion of *CFTR* F508.[3] Differentiation of HL-60 DeltaF508-CF cells into neutrophils occurs upon treatment with DMSO. Differentiated HL-60 DeltaF508-CF cells demonstrate normal levels of CD11b staining but substantially reduced CFTR surface expression compared to differentiated HL-60 control cells, in addition to compromised bactericidal activity.[3] The HL-60 DeltaF508-CF human promyelocytic cell line is the first developed CF myeloid cell line and represents a highly valuable tool for drug screening and investigating the role of phagocytic defects in CF disease onset and progression.

Source

The HL-60 DeltaF508-CF human promyelocytic cell line was derived from HL-60 promyelocytic leukemia cells transfected with a hCFTR-Cas9-eGFP plasmid. Single-cell clones were sorted for eGFP expression and screened for homozygous deletion of *CFTR* F508 via nuclease digestion and DNA sequencing.[3]

Short tandem repeat (STR) Profile

D3S1358: 16	D16S539: 11
TH01: 7, 8	CSF1PO: 13, 14
D21S11: 29, 30	Penta D: 10, 12
D18S51: 14, 15	vWA: 16
Penta E: 13, 14	D8S1179: 12, 13
D5S818: 12	TPOX: 8, 11
D13S317: 8, 11	FGA: 22, 24
D7S820: 11, 12	Amelogenin: X

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from rat, mouse, chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Storage & Handling

HL-60 DeltaF508-CF human promyelocytic cell line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

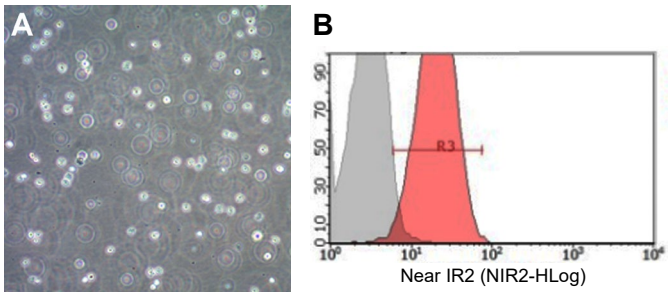

Figure 1. Bright field image of cells (A). FACS analysis of HL-60 DeltaF508-CF cells stained with Rat anti-Mouse CD11b-PE-Cy7 clone M1/70 (B, Cat. No. MABF365). CD11b (red) vs unstained (gray).

References

1. *Nat Rev Dis Primers.* 2015; 1:15010.0
2. *Science.* 2010; 329(5993): 805-810.
3. *J Cyst Fibros.* 2019; 18(1): 44-53.

Rev.1.0/2020-03-09/SCC251/AN

Protocols

HL-60 DeltaF508-CF human promyelocytic cells grow as suspension cells and thus do not require enzymatic detachment or dissociation. Passage when the cell density reaches 1–1.5 million cells/mL. Optimal plating density should be ~200,000 - 250,000 cells/mL. The cells should not be grown at excessively high densities.

1. Do not thaw the cells until the recommended medium is on hand.

   HL-60 DeltaF508-CF Expansion Medium: cells are thawed and expanded in RPMI-1640 (Sigma Cat. No. R0883) supplemented with 2 mM Glutamine (Cat. No. TMS-002-C) and 10% FBS (Cat. No. ES-009-B).

2. Remove the vial of frozen HL-60 DeltaF508-CF cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of HL-60 DeltaF508-CF Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.

6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.

7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 – 20 mL of HL-60 DeltaF508-CF Expansion Medium.
10. Transfer the cell suspension to a T75 flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2. HL-60 DeltaF580-CF suspension cells require media replenishment every 2-3 days. Passage cells when the cell density is at 1 -1.5 million cells/mL.
12. Cells are typically plated at a density of 200,000 - 250,000 cells/mL

Cryopreservation of Cells

HL-60 DeltaF508-CF Human Promyelocytic Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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