CFBE41o- 4.7 ΔF508 CFTR Human CF Bronchial Epithelial Cell Line

SCC159

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Cystic Fibrosis (CF) is a lethal autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene which functions as a cAMP-activated and phosphorylated-regulated Cl channel. The predominant mutation in the CFTR gene is a trinucleotide deletion that results in loss of a phenylalanine at amino acids 508 (ΔF508) in the CFTR protein. This mutation accounts for ~66% of all CF alleles.[1]

CFBE41o- 4.7 ΔF508 CFTR Human CF Bronchial Epithelial Cell line is a subclone derived from the electroporation of the parental CFBE41o- cell line with an Epstein-Barr virus (EBV)-based episomal pCEP4β vector containing the 4.7 kb ΔF508 CFTR open reading frame (ORF) cDNA and a Hygromycin B resistance gene.[1]

The 4.7 kb ΔF508 CFTR cDNA contains the trinucleotide TTT deletion at the ΔF508 locus rather than the naturally occurring CTT and thus makes it possible to differentiate between endogenous ΔF508 CFTR and plasmid derived ΔF508CFTR expression. The parental CFBE41o- is a CF human bronchial epithelial cell line, derived from a CF patient homozygous for the ΔF508 CFTR mutation and immortalized with the origin-of-replication defective SV40 plasmid (pSVori-).[1]

Established CF bronchial epithelial cell lines that are complemented with either wild-type or ΔF508CFTR mRNA will help provide insights into the relationship between transgene-derived CFTR mRNA expression and rescue of cAMP-dependent Cl transport function.

Source

- Human
- COO: United States
- GMO Risk Group: GMO RG2

Short Tandem Repeat

D3S1358	D7S820	vWA	FGA	D8S1179	D21S11	D18S51	D5S818
16, 18	8, 11	17	22, 24	11, 14	29, 31.2	15, 16	11, 13

TH01	TPOX	CSF1PO	AMEL	Penta D	Penta E	D13S317	D16S539
9.3	8, 9	11	X	12	12	9, 11	12

Immortalized 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 ≥ 1 x 10^6 viable cells.
- Cells are tested by PCR and are negative for HPV-16, HPV-18, Hepatitis A, C, and HIV-1 & 2 viruses as assessed by a Human Essential 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 and Handling

CFBE41o- 4.7 ΔF508 CFTR Human CF Bronchial Epithelial Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages without significantly affecting the cell marker expression and functionality.

Representative Data

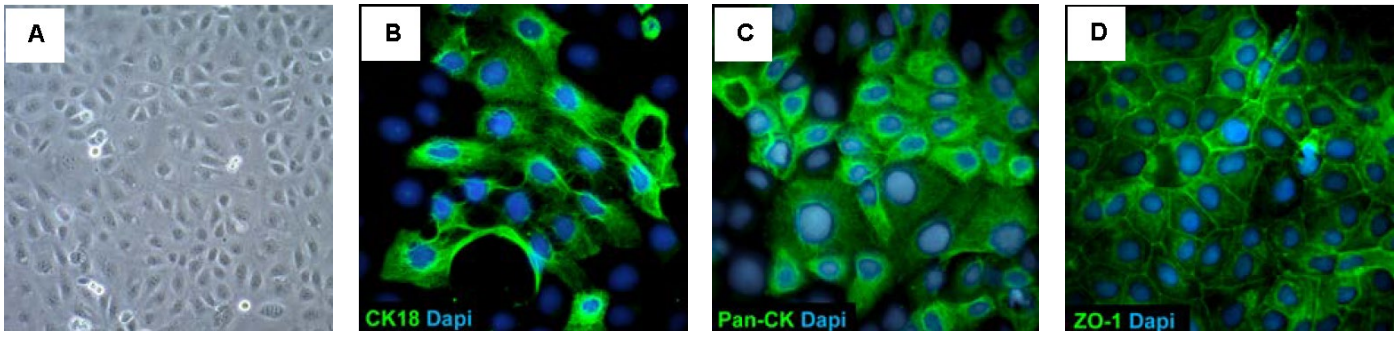

Figure 1. (**A.**) Bright-field image. (**B.** Abcam AB668) Cells express cytokeratin-18, (C. MAB3412) pan-cytokeratin and (**D.** AB2272) the tight junction protein ZO-1.

Protocols

Fibronectin/Collagen/BSA ECM Coating of Flasks

1. Make stock solutions of the following:
   - Human Fibronectin Stock (0.5 mg/mL): Add 10 mL of MEM-Eagle (M2279) to the glass vial containing human fibronectin (F2006-5MG).
   - BSA, Fraction V Stock (1 mg/mL): Weigh out 200 mg BSA (126575) into a 50 mL conical tube. Resuspend BSA in 200 mL 1X PBS or 1X HBS. Sterile filter using a 0.22 µm Stericup® (SCGPU02RE).
   - PureCol™ Collagen Stock (3 mg/mL): Add 5 mL of sterile 0.01 N HCl to 15 mg lyophilized collagen (5006-15MG).

2. Prepare Fibronectin/Collagen/BSA ECM Mixture:

   All products may be purchased from SigmaAldrich.com unless otherwise noted.

Component	Quantity	Final Conc	Catalogue No.
Human Fibronectin Stock (0.5 mg/mL)	2 mL	10 µg/mL	F2006-5MG
BSA, Fraction V Stock (1 mg/mL)	10 mL	100 µg/mL	126575
PureCol® (3 mg/mL)	1 mL	30 µg/mL	5006-15MG
MEM Eagle Medium	87 mL	-	M2279

3. Sterile filter using a 0.22 µm Stericup® (SCGPU02RE). Label and store at 2-8 °C when not in use.
4. Coat flasks with the Fibronectin/Collagen/BSA ECM mixture (3 mL for T25, 6 mL for T75 or 15 mL for T225 flasks). Distribute ECM mixture evenly overgrowth surfaces by swirling. Incubate flasks at room temperature in the hood for at least 2 hours, but no more than 24 hours.
5. Drain coating solution by standing flasks upright for 1-2 minutes. Aspirate. Coated flasks may be stored at room temperature for up to 1 month.
6. Do not rinse flask before use.

Thawing Cells

1. Do not thaw the cells until the recommended medium and ECM coated flasks are on hand.
2. Cells are thawed and expanded in MEM Eagle (M2279), 10% FBS (ES-009-B), 2 mM L-Glutamine (TMS-002-C), 300 µg/mL Hygromycin B (H0654-500MG) and 1X Penicillin-Streptomycin Solution (TMS-AB2-C) (optional).
3. Remove the vial of frozen CFBE41o- 4.7 ΔF508 CFTR Human CF Bronchial Epithelial 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.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. 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.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of 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.
7. 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.
8. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
10. Resuspend the cells in 10-15 mL of Expansion Medium.
11. Transfer the cell mixture to an ECM-coated T75 tissue culture flask.
12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
13. The next day, exchange the medium with 10-15 mL of fresh Expansion Medium. Exchange with fresh medium every other day.

Cell Passage

It is critical to use Trypsin-EDTA solution (T3924). Do not attempt to make your own Trypsin dilution from other sources as it will not work well for the cells. Cells are tightly adherent. Do not use Accutase® or Accumax™ as these are insufficient to detach the cells.

1. Cells are ready to be passaged when they reach 90–95% confluency.
2. Rinse flask twice with 10–15 mL 1X PBS w/o Ca^{2+}, Mg^{2+} (BSS-1006-B). Aspirate after each rinse.
   Note: Be sure to rinse twice to remove residual FBS as cells are very tightly adherent.

3. Add 10 mL Trypsin-EDTA solution (T3924) to the T75 flask. Swirl the flask to ensure that the Trypsin-EDTA completely covers the surface of the flask.
4. Incubate in 37 °C incubator for 7-8 minutes.
5. After 7-8 minutes, take the flask out and for the next 3 minutes, tap firmly on the sides of the flask to dislodge the cells. Total trypsin incubation time is 10 minutes. Do not incubate longer than 10 minutes total.
6. Transfer the dissociated cells to a 50 mL conical tube. Add 15 mL Expansion Medium to the flask to inactivate the trypsin and collect residual cells.
7. Centrifuge at 800-1000 rpm for 3-5 minutes.
8. After centrifugation, discard the supernatant and resuspend the cell pellet in appropriate volume for cell counting.
9. Cells may be passaged using a 1:6 to 1:10 split ratio into the appropriate ECM coated flasks.

Cryopreservation of Cells

CFBE41o- 4.7 ΔF508 CFTR Human CF Bronchial Epithelial Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Illek B, Maurisse R, Wahler L, Kunzelmann K, Fischer H, Gruenert DC. (2008) Cl transport in complemented CF bronchial epithelial cells correlates with CFTR mRNA expression levels. Cell Physiol Biochem 22(1-4): 57-68.

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