CFSMEo- Human Cystic Fibrosis Submucosal Gland Epithelial Cell Line

Immortalized Cell Line

Cat. # SCC155

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THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

Data Sheet

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. In CF, altered Cl- transport and secretion results in the production of thick and viscous mucus that can damage many of the body's organs. Tracheobronchial submucosal glands secrete mucins and antimicrobial substances that keep the airways sterile along with fluids that help hydrate airway surfaces. The relationship between CF and mucus secretion is unclear and require further investigations[1,2].

CFSMEo- is a human tracheobronchial submucosal gland epithelial cell line isolated from an individual with CF, who was compound heterozygote for the ΔF508 and Q2X CFTR mutations[1]. ΔF508 mutation 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]. Q2X mutation is a rare CF mutation in exon 1 of the CFTR gene in which the second codon (CAG) is mutated into the stop codon UAG. The CFSMEo- cell line is the result of pooled colonies that arose from immortalization of the human CF tracheobronchial submucosal gland cells with the origin-of-replication defective SV40 plasmid (pSVori-)[1,2].

CFSMEo- retains the characteristic cobblestone morphology of epithelial cells along with cytokeratin expression and the ability to form tight junctions. The cell line expresses vestigial amounts of CFTR mRNA transcripts but does not express detectable levels of CFTR protein[1]. CFSMEo- lacks cAMP-induced Cl- currents[2].

Quality Control Testing

- Each vial contains ≥ $1X10^6$ 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.

Short tandem repeat (STR) Profile

D3S1358: 18	D16S539: 9, 14
TH01: 7, 9.3	CSF1PO: 12
D21S11: 29, 30	Penta D: 9, 10
D18S51: 16	vWA: 14, 17
Penta E: 10, 11	D8S1179: 8, 13
D5S818: 12	TPOX: 8, 11
D13S317: 12	FGA: 19
D7S820: 9, 11	Amelogenin: X

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.

Storage and Handling

CFSMEo- Human CF Submucosal Gland Epithelial 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.

Representative Data

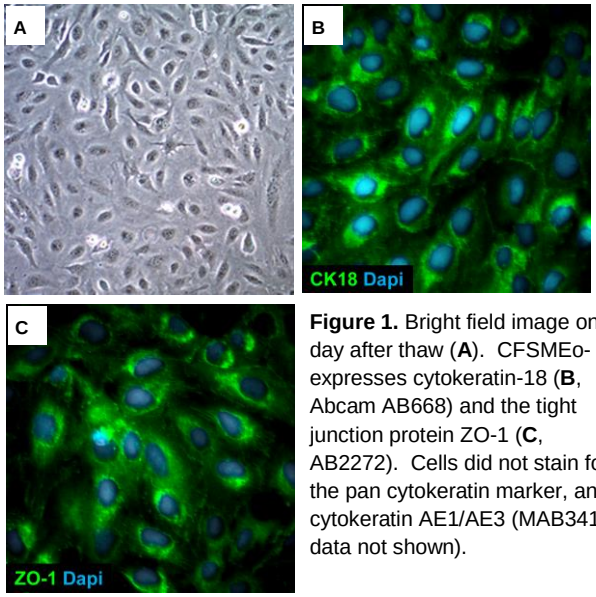

Figure 1. Bright field image one day after thaw (**A**). CFSMEo- expresses cytokeratin-18 (**B**, Abcam AB668) and the tight junction protein ZO-1 (**C**, AB2272). Cells did not stain for the pan cytokeratin marker, anti-cytokeratin AE1/AE3 (MAB3412, data not shown).

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Protocols

Fibronectin/Collagen/BSA ECM Coating of Flasks

1. Make stock solutions of the following:

   a) Human Fibronectin Stock (0.5 mg/mL): Add 10 mL of α-MEM (Sigma M2279) to the glass vial containing human fibronectin (Sigma F2006-5MG).

   b) BSA, Fraction V Stock (1 mg/mL): Weigh out 200 mg BSA (Cat. No. 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 (Cat. No. SCGPU02RE).

   c) PureCol Collagen Stock (3 mg/mL): Add 5 mL of sterile 0.01N HCL to 15 mg lyophilized collagen (Sigma 5006-15MG).

2. Prepare Fibronectin/Collagen/BSA ECM Mixture.

Component	Quantity	Final Conc.	Supplier	Cat #
Human Fibronectin Stock (0.5 mg/mL)	2 mL	10 μg/mL	Sigma	F2006-5MG
BSA, Fraction V Stock (1 mg/mL)	10 mL	100 μg/mL	EMD Millipore	126575
PureCol (3 mg/mL)	1 mL	30 μg/mL	Sigma	5006-15MG
α-MEM Medium	87 mL	NA	Sigma	M2279

3. Sterile filter using a 0.22 μm SteriCup (Cat. No. 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 over growth 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.

   Cells are thawed and expanded in α-MEM (Sigma Cat. No. M2279), 10% FBS (Cat. No. ES-009-B) and 2 mM L-Glutamine (Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C) (optional).
2. Remove the vial of frozen CFSMEo- 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 CFSMEo- 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 10 -15 mL of CFSMEo- Expansion Medium.

10. Transfer the cell mixture to an ECM-coated T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh CFSMEo- Expansion Medium. Exchange with fresh medium every other day.

Cell Passage

Note: It is critical to use Sigma T3924 Trypsin-EDTA solution. 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 Ca2+, Mg2+ (Cat. No. 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 (Sigma 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 = 10 minutes. **Do not incubate longer than 10 minutes total.**
6. Transfer the dissociated cells to a 50 mL conical tube. Add 15 mL CFSMEo- 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

CFSMEo- Human CF Submucosal Gland Epithelial Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Cozens AL, Yezzi MJ, Chin L, Simon EM, Finkbeiner WE, Wagner JA, Gruenert DC. (1992) Characterization of immortal cystic fibrosis tracheobronchial gland epithelial cells. Proc Natl Acad Sci USA 89(11): 5171-5175.
2. da Paula AC, Ramalho AS, Farinha CM, Cheung J, Maurisse R, Gruenert DC, Ousingsawat J, Kunzelmann K, Amaral MD. (2005) Characterization of novel airway submucosal gland cell models for cystic fibrosis studies. Cell Physiol Biochem 15(6): 251-262.

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