16HBE14o- Human Bronchial Epithelial Cell Line

Immortalized Cell Line

Cat. # SCC150

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.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Data Sheet

Background

16HBE14o- is a human bronchial epithelial cell line isolated from a 1-year old male heart-lung patient and immortalized with the origin-of-replication defective SV40 plasmid (pSVori-). The cell line retains characteristic features of normal differentiated bronchial epithelial cells including a cobblestone morphology, cytokeratin expression, the ability to form tight junctions, and directional ion transport[1]. When grown with an air/liquid interface, cilia can be detected. In contrast to most other respiratory cell lines, 16HBE14o- expresses high levels of cystic fibrosis transmembrane conductance regulator (CFTR) mRNA and protein[1]. Expression of CFTR is correlated to cAMP-dependent Cl- conductance in a variety of cells, including 16HE14o- epithelial cells.

16HBE14o- is widely used to model barrier function of the airway epithelium in vitro and to study respiratory ion transport as well as the function of CFTR.

Short tandem repeat (STR) Profile

D3S1358: 16	D16S539: 12, 13
TH01: 9.3	CSF1PO: 11
D21S11: 28, 30.2	Penta D: 9
D18S51: 14	vWA: 16, 18
Penta E: 5, 14	D8S1179: 10, 11
D5S818: 11, 12	TPOX: 8
D13S317: 11	FGA: 21, 22
D7S820: 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.

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.

Storage and Handling

16HBE14o- Human Bronchial 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

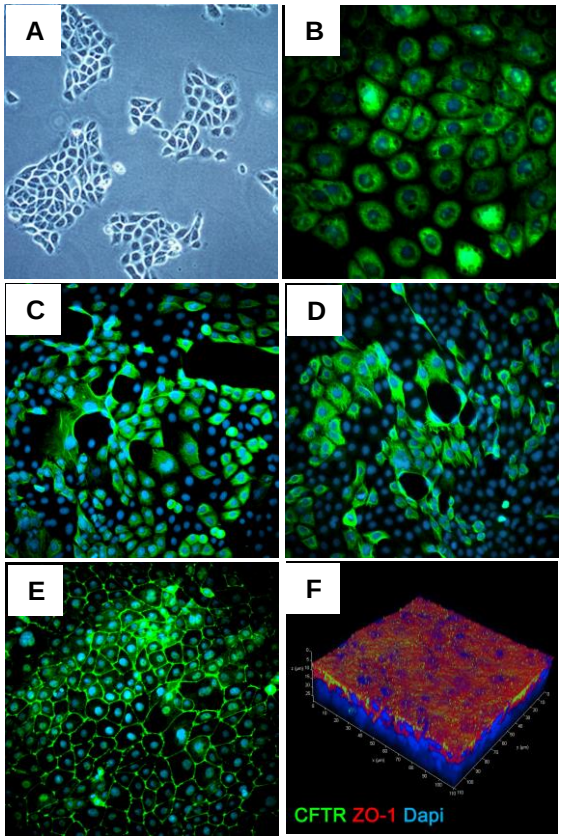

Figure 1.
16HBE14o- cells one day after thaw (**A**). 16HBE14o- expresses CFTR (**B**, MAB3484), cytokeratin-18 (**C**, Abcam AB668), pan-cytokeratin (**D**, MAB3412) and the tight junction protein ZO-1 (**E**, AB2272). 16HBE14o- cultured on air/liquid interface retained polarity as shown by apical immunostaining of CFTR (**F**, green).

Rev.1.0/2018-03-12/SCC150/AN

Please visit www.millipore.com for additional product information and references.
Submit your published journal article, and earn credit toward future purchases. Visit www.millipore.com/publicationrewards to learn more!

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 16HBE14o- 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 16HBE14o- 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 16HBE14o- 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 16HBE14o- Expansion Medium. Exchange with fresh medium every other day.

Cell Passage

Note: It is absolutely 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 16HBE14o- 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

16HBE14o- Human Bronchial 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, Kunzelmann K, Ohrui T, Chin L, Eng K, Finkbeiner WE, Widdicombe JH, Gruenert DC. (1994) CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol*. 10(1): 38-47.
2. Kunzelmann K, Koslwosky T, Hug T, Gruenert DC, Greger R. (1994) cAMP-dependent activation of ion conductances in bronchial epithelial cells. *Pflugers Arch* 428(5-6): 590-596.
3. Koslowsky T, Hug T, Ecke D, Klein P, Greger R, Gruenert DC, Kunzelmann K. (1994) Ca(2+)- and swelling-induced activation of ion conductances in bronchial epithelial cells. *Pflugers Arch* 428(5-6): 597-603.
4. Wan H, Winton HL, Soeller C, Stewart GA, Thompson PJ, Gruenert DC, Cannell MB, Garrod DR, Robinson C. (2000) Tight junction properties of the immortalized human bronchial epithelial cells Calu-3 and 16HBE14o-. *Eur Respir J.* 15(6): 1058-1068.
5. Illek B, Lei D, Fischer H, Gruenert DC. (2010) Sensitivity of chloride efflux vs. transepithelial measurements in mixed CF and normal airway epithelial cell populations. *Cell Physiol Biochem* 26(6): 983-990.

ACADEMIC USE AGREEMENT
(subject to local law)

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means 16HBE14o- Human Bronchial Epithelial Cell Line (SCC150)

"Academic Research" means any internal *in vitro* research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.