Description

The CFTR HEK293 Cell Line expresses full length, wild-type human cystic fibrosis transmembrane conductance regulator (CFTR) protein (Genbank #P13569, NP_000483.3), with a C-terminal Streptavidin-Binding Peptide (SBP) tag.

Expression must be induced ≥24 hours prior to an experiment using 1 µg/ml Doxycycline and 3 mM Na-butyrate. The inducible expression of CFTR was confirmed by Western blotting and flow cytometry.

Background

Cystic fibrosis transmembrane conductance regulator (CFTR) is a protein that in humans is encoded by the CFTR gene. CFTR is an ABC transporter-class ion channel that transports chloride and thiocyanate ions across epithelial cell membranes. Mutations of the CFTR gene affect functioning of the chloride ion channels in these cell membranes, leading to cystic fibrosis. It is characterized by the triad of chronic bronchopulmonary disease (associated with recurrent respiratory infections), pancreatic insufficiency, which leads to malabsorption and growth retardation, and elevated sweat electrolytes.

Application

- Compound or antibody screening
- Functional assays
- Antigen preparation for mouse immunization

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

293HEK-Trex, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 4	BPS Bioscience #60181
Growth Medium 4A	BPS Bioscience #79535

Materials Required for Induction of CFTR Protein Expression

Name	Ordering Information
Growth Medium 4C	BPS Bioscience #79631

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37˚C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 4 (BPS Bioscience #60181):
DMEM/F12 (1:1) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 4A (BPS Bioscience #79535):
DMEM/F12 (1:1) supplemented with 10% FBS, 1% Penicillin/Streptomycin, 10 µg/ml Blasticydin, 200 µg/ml Zeocin.

Media Required for Induction of Target Protein Expression

Growth Medium 4C (BPS Bioscience #79631):
DMEM/F12 (1:1) supplemented with 10% FBS, 1% Penicillin/Streptomycin, 1 µg/ml Doxycycline, and 3 mM Na-butyrate.

Culture cells in induction medium for 24 hours prior to the experiment.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 4 **(no Blasticydin or Zeocin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 4 **(no Blasticydin or Zeocin)**.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 4 **(no Blasticydin or Zeocin)** and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 4A **(contains Blasticydin and Zeocin)**.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 4 and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 4A **(contains Blasticydin and Zeocin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:5 to 1:10 weekly or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

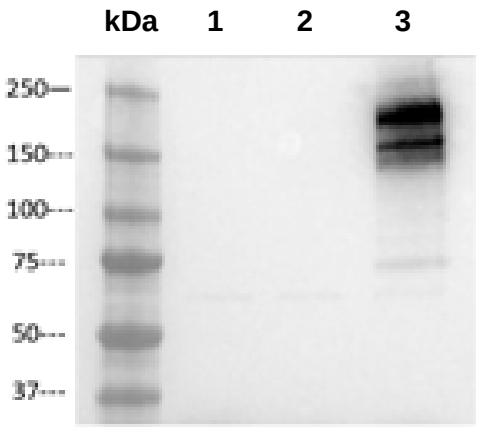

Figure 1. Western Blot of the CFTR expressing cells.
CFTR HEK293 cells were induced with 1 µg/ml Doxycycline and 3 mM Na-butyrate for 24 hours prior to cell lysis using 50 mM Tris-HCl, 150 mM NaCl, 0.5% Igepal and 1x HALT protease inhibitor cocktail. CFTR expression was analyzed by western blot using an anti-CFTR antibody (MSM Protein Technologies). The protein ladder is shown on the left. Lane 1: parental HEK293-Trex cells Lane 2: Uninduced CFTR HEK293 cells. Lane 3: induced CFTR HEK293 cells.

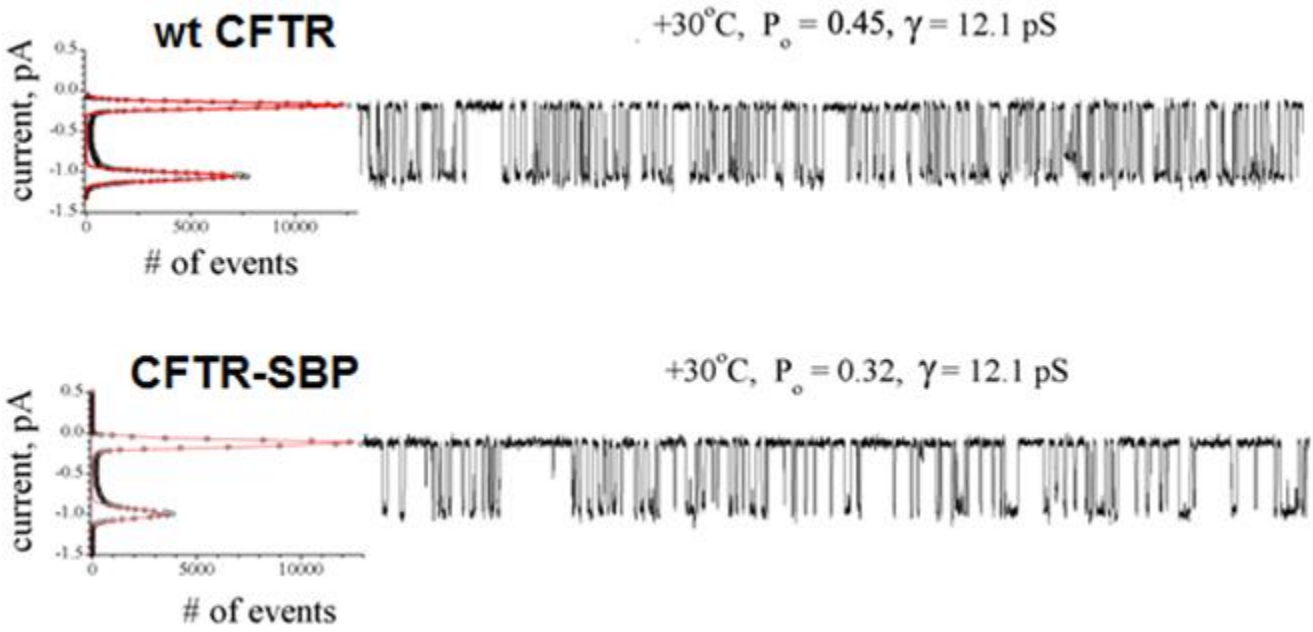

Figure 2. Electrophysiological analysis of CFTR cells.
Comparison of endogenous CFTR versus recombinant CFTR-SPB.

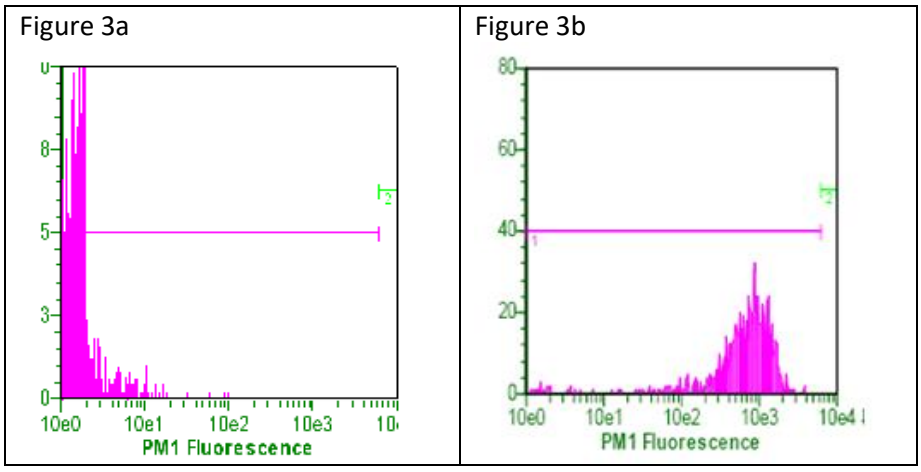

Figure 3. CFTR expression profile by flow cytometry.
CFTR expression on cell surface measured by flow cytometry using intracellular cell staining by Streptavidin-PE. Figure 3a: Host cells, Figure 3b: CFTR expressing cells.

Sequence
Wild-type human CFTR protein with a C-terminal Streptavidin-Binding Peptide (SBP) tag stably integrated in 293HEK-Trex cells (Genbank #P13569, NP_000483.3)

References
1. Gadsby, D.C., et al. Nature **440** (7083): 477–483 (2006).
2. Hillier, L.W., et al. Nature **424**:157-164 (2003).
3. McCann, C. M., et al. BioTechniques **38** (6): 945–952 (2005).

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
GPRC5D HEK293 Cell Line	78345	2 vials
CGRPR/CRE Luciferase Reporter HEK293 Cell Line	78325	2 vials
GLP-1R/CRE (Luc) Reporter – HEK293 Recombinant Cell Line	78176	2 vials
Adenosine A2A Receptor Functional Recombinant Cell Line	79381	2 vials