MANUAL

HLA-A:02:01 Stably expressing CFBE (WT-CFTR) Cell Line

Catalog #: HLA-0007

ISO 13485

Introduction

CFBE is a Cystic Fibrosis (CF) human bronchial epithelial cell line, derived from a CF patient homozygous for the ΔF508 CFTR mutation. CFBE cells can polarize and form tight junction. They demonstrate all ion transport properties characteristic of cystic fibrosis, such as defective cAMP-dependent chloride transport and intact calcium dependent chloride transport. The CFBE cell line subclones, WT-CFTR and ΔF508-CFTR, can be used to study the relationship between CFTR gene expression and chloride transport function.

CFBE-HLA-A:02:01 stable cell line is transformed from the CFBE (WT-CFTR) cell line and stably expresses the HLA-A:02:01 protein. The expression of HLA-A:02:01 has been validated by western blot.

Protein Accession Number: P04439.

Provided Materials

One vial of 2 x 10^6 cells, at P4 in Freezing Media.
IMPORTANT: store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Additional Materials Required

1. Minimum Essential Medium α (MEM α)
2. Fetal Bovine Serum (FBS)
3. Penicillin/Streptomycin
4. Trypsin
5. Phosphate-buffered saline (PBS)
6. DMSO
7. 96-well white plate

Handling Cells Upon Arrival

It is strongly recommended that you propagate the cells following instructions as soon as possible upon arrival.
IMPORTANT: An adequate number of frozen stocks must be made from early passages as cells will undergo genotypic changes. Genetic instability in transfected cells will result in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

Complete Growth Media

In 450mL of MEM α, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).

Freezing Media

Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Initial Culture Procedure

1. Quickly thaw cells in a 37 °C water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Transfer cells to a 15ml centrifuge tube containing 7ml of pre-warmed Complete Growth Media.
3. Centrifuge tube at 1200-1500 RPM for 5 minutes.
4. Remove supernatant and resuspend cells with 1ml Complete Growth Media.
5. Transfer cells to a T75cm^2 tissue culture flask or 100 mm culture dish containing 8-12ml of Complete Growth Media.
6. Place the flask with cells in a humidified incubator at 37 °C with 5% CO_2

Subculture Procedure

A sub-cultivation ratio of 1:3 to 1:4 is recommended with media changes every 2 to 3 days.

Preparing Frozen Stocks

This procedure is designed for 60mm^2 dish or T25cm^2 flask. Scale volumes according to other vessels.

1. When cells reach 1-1.5x10^6/ml, freeze down cells.
2. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 1200-1500 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media and resuspend cells in 1ml freezing media and gently resuspend by pipetting up and down.
4. Transfer 1mL of cells into a cryogenic vial.
5. Place the cryogenic vial in a freezing container and store it at -80 °C freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

This product is for research use only.