16HBE14o- (WT CFTR) Cell Line Notes

Parental 16HBE14o- cells were generated by Dieter Grünert (UCSF) and obtained under MTA.

I. Basic Cell Culture

Medium (Store complete medium at 4°C)

Component	CFF Vendor/Catalog	% (final)
Minimum Essential Medium	Gibco 11095-072	89%
Fetal bovine serum	Gibco 26140-079	10%
Penicillin/Streptomycin (100x)	Gibco 15140-122	1%

Freezing Solution (Make fresh)

Component	% (final)
Complete media (see above)	40%
Fetal bovine serum	50%
DMSO	10%

Other Reagents
DPBS (Hyclone SH30028.02)
TrypLE Express (Gibco 12604-021)

Coating Solution (Make fresh)

Component	CFF Vendor/Catalog	50mL final solution
LHC-8 basal medium	Gibco 12677-027	48 mL
Bovine serum albumin 7.5%	Gibco 15260-037	67 µL
Bovine collagen solution, Type 1	Advanced BioMatrix 5005-100ML	0.5 mL
Fibronectin from human plasma, 1mg/ml	Thermo Fisher Scientific 33016-015	0.5 mL

- Coat flasks with the coating solution: 1ml of the solution for a T-25 flask, 2ml for a T-75. Distribute the solution evenly across the surface, making sure the entire surface is wetted by the solution and leave for 2-3 hours at 37°C. After incubation, thoroughly remove liquid. Do not reuse this solution. Do not rinse the containers. The coated flasks can be stored at 4°C for several months.

Sub-Culturing

- Sub-culture at 80-100% confluence
- Remove medium and rinse with DPBS and remove
- Add TrypLE Express (spread across the cell surface) and incubate the flask at 37°C
- Monitor cell detachment under the microscope
- Once cells are detached, add complete media.
- Pipette the cells repeatedly to break up any clumps and transfer cell suspension into a centrifuge tube.

- Spin for 3.5 minutes at 1200 rpm to pellet the cells
    - Note: If you cannot spin the cells (such as high throughput 96 well format), this step can be skipped
- Discard the liquid and resuspend the cell pellet in complete medium.
- Split cells at 1:10 to 1:20 for one week passages
    - CFF media change schedule – 3x weekly

Freezing down the cells for liquid nitrogen storage

- Treat the cells as for sub-culturing except resuspend the cell pellet in freezing solution.
- Transfer the cells into freezing vials, 1-1.5mL/vial
- Put the vials in controlled colling chamber and plate in -80^{o}C freezing overnight (the chamber regulates cooling at ~1^{o}C/minute)
- Move the vials from the cooling chamber into liquid nitrogen storage as quickly as possible

Culturing cells from frozen vials

- Remove a vial of cells from liquid nitrogen and thaw the cells in a 37^{o}C water bath
- Resuspend with 5 mL complete medium
- Spin for 3.5 minutes at 1200 rpm to pellet the cells
- Remove liquid (to remove DMSO)
- Resuspend cell pellet with 9 mL medium (for T-75 flask) and see the cells onto a coated flask
- Grow cells in 37^{o}C incubator with 5% CO_2.
- After overnight incubation, the cells should attach to the flask. The rounded, un-attached cells are dead cells.