# Culture of 16HBE14o- Cells

**Nicole A. McNabb**
US Environmental Protection Agency
**Shaun D. McCullough** ( mccullough.shaun@epa.gov )
US Environmental Protection Agency https://orcid.org/0000-0001-6660-346X

**Method Article**

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# Abstract

This protocol describes the thawing, culturing, and cryopreservation of the human bronchial epithelial cell line 16HBE14o- (referred to as “16HBE”). The attached methods document is a formal version of the information included here. The attached worksheet is a fillable PDF that can be used to maintain cell passage records using this protocol.

Please note: Deviation from the three-day passage cycle and cell plating density described here typically results in greater culture and experimental variability.




# Reagents

Minimum Essential Medium (MEM) with Earle’s salts and L-glutamine (Gibco #11095)

Fetal bovine serum (Gibco #16000-044)

100X penicillin/streptomycin solution (Gibco #15140-122)

Dulbecco’s Phosphate Buffered Saline (DPBS, Gibco #14190-144)

Trypsin (Gibco #25200-056)

Dimethylsulfoxide (DMSO, Sigma #D8418)

Isopropanol (Sigma #H9516)

# Equipment

Biosafety cabinet

Humidified tissue culture incubator with 5% $CO_2$

Tissue culture dishes

100mm plates (TPP #93100)

150mm plates (TPP #93150)

Vacuum-driven bottle filter with 0.2 µm pore

Sterile 125 mL and 250 mL bottles

Pipetaid

Serological pipettes

50mL conical tubes

Tabletop centrifuge

Pipettes

Filter tips (low retention)

- P1000 (BioExpress #P-1237-1250)
- P200 (BioExpress #P-1237-200)
- P20 (BioExpress #P-1237-20)
- P2 (BioExpress #P-1237-10XL)

Hemocytometer or automated cell counting device

“Mr. Frosty” cell freezing apparatus (Nalgene; Fisher #5100-0001)

Long-term cell storage container with liquid nitrogen

# Procedure

## Prepare Culture Medium

1. Remove 50mL of volume from the bottle of MEM basal medium and discard.
2. Add 50mL of fetal bovine serum (FBS) to the remaining volume of MEM.
3. Add 5mL of 100X penicillin/streptomycin to the medium.
4. Mix thoroughly and label with the date and additives.

## Collagen Coating of Tissue Culture Plates

16HBE cells should be grown on dishes/plates/inserts that have been coated with 50 mg/mL bovine collagen I solution according to the separate method “Collagen Coating for Tissue Culture”.

## Thawing Cryopreserved Cells

1. Warm media and prepare materials prior to obtaining a vial of cells from cryostorage.

a. This reduces the amount of time cells are thawed in the presence of DMSO.

2. Remove vial from liquid nitrogen (wearing eye protection) and thaw in 37°C water bath (1-2 min).

a. It is important that cells are thawed quickly to prevent damage and reduced viability.

3. Add 24 mL of growth medium to a 50 mL conical tube then add the thawed cells and gently mix by inversion.

4. Pellet cells via centrifugation at 1,000 x *g* for 3 minutes at room temperature.

5. Carefully aspirate the supernatant.

6. Gently resuspend the cells in 10mL of pre-warmed growth medium and transfer into a 10 cm collagen coated tissue culture dish (see separate method “Collagen Coating for Tissue Culture”).

7. Incubate in a humidified 37 °C incubator with 5% $CO_2$ overnight.

8. Check cells the following day for attachment to the dish. Replace medium to remove dead cells (rounded, unattached cells). Culture at least three passages before using in experiments.

## Sub-culturing cells

Cells should be split three days after being plated at 2.5 x $10^5$ cells/mL

1. Pre-warm growth medium and PBS in a 37 °C water bath and a trypsin aliquot at room temperature (~30 minutes).

2. Aspirate the cull culture medium.

3. Rinse cells in 10 cm plates with 10 mL pre-warmed PBS (use 20 mL for 15 cm plates), swirling gently.

4. Aspirate the PBS wash.

5. Add 750 µL trypsin (for 10 cm plate, 1.5mL of 15 cm plate) and spread evenly across the cell surface

6. Place in 37 °C incubator for 4 minutes. Following incubation, use the palm of your hand to hit the side of dish to facilitate detachment. If cells are still attached, incubate for another 2 minutes. Incubate

and tap dish until >95% of the cells are detached. Check detachment with microscope.

7. Add 12 mL medium and gently wash the surface of the dish with a pipette and triturate once to break up any large cell clumps. Transfer to a 50 mL centrifuge tube.

8. Wash the plate with an additional 12 mL of medium, collect, triturate, and add to the cell suspension in step #7.

9. Centrifuge at 1,000 x *g* for 4 minutes to pellet cells.

10. Aspirate the supernatant and resuspend in 12 mL of medium. Add additional growth medium to dilute the cell suspension if desired. Typically, medium is added such that there is a total volume of 12 mL per 1-2 15 cm plates collected.

11. Prepare a 1:5 dilution in PBS (800 µL PBS + 200 µL cell suspension) in an Eppendorf tube.

12. Count cells on a hemocytometer (do not use an automated cell counter as these cells tend to form small clumps that are not accurately quantified by automated methods).

13. Dilute cells to 2.5 x $10^5$ cells/mL in growth medium in a 50 mL tube or larger sterile bottle to the total volume that will be used for plating (10 mL for each 10 cm plate and 25 mL for each 15 cm plate).

14. Mix thoroughly by swirling and dispense the diluted cell suspension into collagen coated tissue culture dishes (see separate method “Collagen Coating for Tissue Culture”).

15. Incubate in a humidified incubator at 37 °C with 5% $CO_2$. Cells will be ready to split three days after plating.

a. **NOTE:** plating cells at a determined cell density will result in more predictable sub-culture and more reproducible performance in subsequent experimental assays.

## Preparing cells for cryopreservation

1. Prepare freezing medium.

a. 50% FBS, 40% growth medium, 10% DMSO.

b. Filter through a 0.2 µm syringe filter.

2. Follow sub-culturing protocol through step #7.

3. Determine the number of cells present and pellet cells by centrifugation at 1,000 x *g* for 3 minutes at room temperature.

4. Resuspend the cell pellet at a density of 3.0 x $10^6$ cells/mL in freezing medium.

5. Aliquot into cryovials (1 mL per vial).

6. Put vials in a controlled cell freezing container (filled to indicated line with isopropyl alcohol) and place in a -80 °C freezer overnight.

a. The chamber regulates cooling to about 1 °C per minute.

b. NOTE: isopropanol should be changed after every three freezing cycles.

c. NOTE: do not leave vials at -80 °C longer than overnight as it will impact viability after thawing.

7. Put the frozen vials in liquid nitrogen storage.

## Troubleshooting

## Time Taken

## Anticipated Results

## References

Original description of cells:

Cozens AL, *et al.* (1994) CFTR expression and chloride secretion in polarized immortal human bronchial epithelial cells. *Am J Respir Cell Mol Biol*. 10(1): 38-47

https://www.ncbi.nlm.nih.gov/pubmed/7507342

## Acknowledgements

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- McCulloughMethodsCutureof16HBE14oCellsR1.docx
- 16HBE14oCellCultureWorksheet.pdf