EpiX™ Epithelial Cell Expansion Protocol

Catalog # 276-201

Description

The EpiX™ medium is a serum-free medium and supports trillion-plus fold expansion of skin, airway, mammary and other epithelial cells without the use of feeder cells. EpiX medium is compatible with standard genetic engineering procedures and supports cloning out single cells. To formulate EpiX medium, add the provided supplements (Cat Nos. 276-00A, 276-00B, 276-00C) into the base medium (Cat No. 276-00D). EpiX medium does not contain antibiotics.

Intended Use

For Research Use Only.

Storage

1. Check all containers for leakage or breakage.
2. Store the EpiX medium supplement kit below -20°C, and avoid repeated freeze/thaws.
3. Store the EpiX base medium at 2 – 8°C.
4. After adding the supplements to base medium, store the complete EpiX medium at 2 – 8°C and use within one month.

Usage Protocol

This protocol describes the use of EpiX medium to expand human epithelial cells. At the start of the culture, thaw epithelial cells, count, and plate the cells onto collagen I coated tissue culture vessels. Change the EpiX medium every 2 – 3 days until the cells approach 80% – 90% confluence, when they are passaged into new culture vessels. Continue the process until the desired population doublings (PDs) or the desired biomass has been achieved.

Population doubling: Population doubling (PD) refers to the total number of times the cells in the population have doubled during the culture period. Use the following equation to determine the number of population doublings.

$$PD = 3.32 \quad x \quad log\left(\frac{[total\ cell\ number\ at\ harvest]}{[total\ viable\ cell\ number\ seeded\ at\ the\ beginning\ of\ culture]}\right)$$

Reagents required

1. Human epithelial cells for expansion.
2. Collagen I coated culture vessels. e.g., Corning® BioCoat™ Cellware, Collagen Type I, T-25 flask (Cat # 356484), or Corning® BioCoat™ Cellware, Collagen Type I, T-75 flask (Cat# 356485).
3. 0.05% Trypsin-EDTA, e.g., Thermo Fisher Scientific, Cat# 25300-054.
4. Ca^{2+}, Mg^{2+} free PBS, e.g., Thermo Fisher Scientific, Cat# 10010-023.
5. 4% (v/v) FBS in PBS. Dilute FBS (e.g., Thermo Fisher Scientific Cat# 26140-079) in PBS.
6. DMSO for cell cryopreservation, e.g., Sigma, Cat# D2650.
7. CoolCell® Cryopreservation Alcohol-Free Cell Freezing Containers, BioCision®.

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Procedure

I. Preparation of EpiX medium

1. Thaw the EpiX medium supplement kit at room temperature. Add the following to each bottle (500 ml) of EpiX base medium to make complete medium:
    a. 100 µl supplement A
    b. Whole contents of supplement B vial
    c. 100 µl supplement C
2. Mix thoroughly by gently swirling. Store at 2 – 8°C until needed.

II. Thawing frozen epithelial cells

1. Thaw epithelial cells and initiate cell culture as soon as possible upon receiving the frozen cells. If not to be used right way, the cells should be stored in liquid nitrogen vapor phase.
2. Immediately prior to use, thaw the vial of epithelial cells by gentle agitation in a 37°C water bath. Thawing should be complete in approximately 2 – 3 minutes.
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate it by spraying with 70% ethanol.
4. Transfer the vial contents to a 15 ml conical centrifuge tube containing 5 ml of warm EpiX medium. Centrifuge the cell suspension at approximately 200 x g for 5 minutes.
5. Aspirate most of the supernatant from the conical centrifuge tube containing the cell pellet. Flick the tube briefly to loosen the pellet, and then resuspend the cells in 0.5 – 1 ml warm EpiX medium.
6. Determine the viable cell number using an automated cell counter such as Countess II or a hemocytometer using standard Trypan Blue exclusion assay.
7. Seed epithelial cells between 1,000 – 5,000 viable cells/cm^2. Mix the desired numbers of epithelial cells with the EpiX medium to ensure a uniform suspension, and dispense the calculated volume into the culture flask. Use 1 ml EpiX medium for every 5 cm^2 surface area, e.g., use 5 ml EpiX medium for a 25 cm^2 flask.

    Note: Early-passage epithelial cells may have a doubling time of less than 24 hours in EpiX medium and may need to be split every 4 – 5 days. After 8 – 10 passages, the growth of epithelial cells may slow down and need to be split every 6 – 7 days.

8. Incubate the cultures at 37°C in a 5% CO_2 humidified incubator.
9. Change the growth medium every 2 – 3 days. As the cells become more confluent, increase the volume of medium as follows: under 50% confluence feed 1 ml per 5 cm^2, over 50% confluence feed 1.5 ml per 5 cm^2.

III. Sub-culture epithelial cells culture

The epithelial cells culture should be split when the cells approach 80% – 90% confluence. **DO NOT** allow the cells to reach complete confluence, as this will induce the cells to differentiate and significantly decrease their ability to continuously replicate.

Volumes used in this protocol are for a 25 cm^2 flask; proportionally reduce or increase the amount of media for culture vessels of other sizes.

1. Remove spent medium.
2. Add 3 – 5 ml PBS to the flask. Briefly rinse the cells and discard the rinse solution.
3. Add 1 ml warm 0.05% Trypsin-EDTA to the flask. Incubate at 37°C for 5 min, or longer time if necessary, to detach the cells. Tap the flask several times against your palm to loosen the cells.
4. Neutralize the trypsin by adding 3 – 4 ml warm 4% FBS in PBS, and pipette up and down several times to break up any cell clumps into suspension.
5. Transfer the cell suspension into a sterile 15 ml centrifuge tube. Rinse the flask with an additional 1 – 2 ml 4% FBS in PBS and pool into the centrifuge tube with cells.
6. Centrifuge cells at 200 x g for 5 min at room temperature.
7. Aspirate most of the supernatant from the centrifuge tube containing the cell pellet. Flick the tube briefly to loosen the pellet.
8. Dilute the cells in 1 ml of warm EpiX medium. Determine cell count and viability using an automated cell counter such as Countess II, or a hemocytometer using Trypan Blue.
9. Use the following equation to determine the number of population doubling (PD).

$$PD = 3.32 \quad x \quad \log\left(\frac{[total\ cell\ number\ at\ harvest]}{[total\ viable\ cell\ number\ seeded\ at\ the\ beginning]}\right)$$

10. Seed between 1,000 – 5,000 viable cells/cm^2 into a new subculture vessel(s). Mix desired number of epithelial cells in the EpiX medium to ensure a uniform suspension, and dispense the calculated volume into the subculture flasks. Use 5 ml EpiX medium for a 25 cm^2 flask.

    Note: Early-passage epithelial cells may have a doubling time less than 24 hours in EpiX medium and may need to be split every 4 – 5 days. After 8 – 10 passages, the growth of epithelial cells may slow down and need to be split every 6 – 7 days.

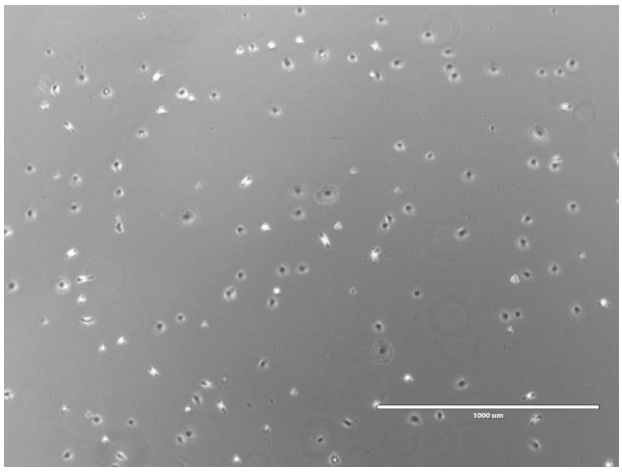
11. Incubate the cultures at 37°C in a 5% CO_2 humidified incubator.
12. Change the growth medium every 2 – 3 days. As the cells become more confluent, increase the volume of media as follows: under 50% confluence feed 1 ml per 5 cm^2; over 50% confluence feed 1.5 ml per 5 cm^2.

IV. Cryopreservation of epithelial cells

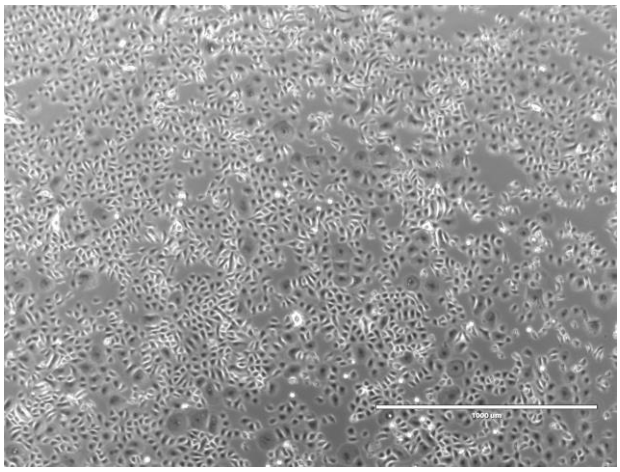
1. Cryopreservation Medium: EpiX medium, 80% (v/v); FBS, 10% (v/v); DMSO, 10% (v/v).
2. Follow the subculture protocol to harvest epithelial cells when they approach 80% – 90% confluence.

3. Determine cell count and viability using an automated cell counter such as Countess II or a hemocytometer using Trypan Blue.
4. The cells can be frozen at a density of 0.5 – 5 x 10^6 cells/ml. Resuspend cells in an appropriate amount of cryopreservation medium, and aliquot into cryovials.
5. Put the cryovials into a CoolCell™ and leave it in a -80°C freezer for at least 2 hours or overnight.
6. Transfer the cells to the vapor phase of liquid nitrogen for long-term storage.

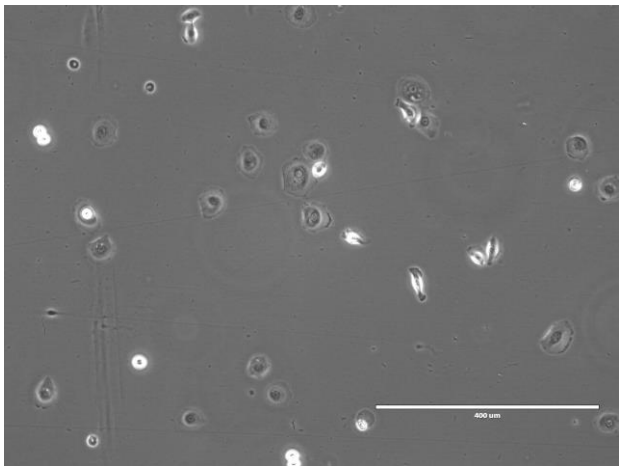
Examples of bronchial epithelial cell culture



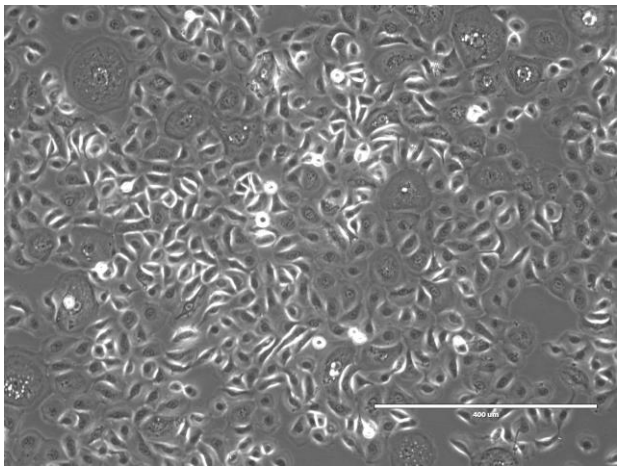
Day 1 culture of human bronchial epithelial cells in EpiX medium, seeded at 3,000 cells/cm2. Photo is taken at 4X.



Day 4 culture of human bronchial epithelial cells in EpiX medium, seeded at 3,000 cells/cm2. Photo is taken at 4X. The cells should be split.



Day 1 culture of human bronchial epithelial cells in EpiX medium, seeded at 3,000 cells/cm^2. Photo is taken at 10X.



Day 4 culture of human bronchial epithelial cells in EpiX medium, seeded at 3,000 cells/cm^2. Photo is taken at 10X. Majority of the cells are small in size and exhibit high nucleus/plasma ratio, although a small percentage of cells look much bigger and may contain many vesicles.

Quality Assurance

1. EpiX medium is tested prior to shipment to meet quality control specifications. Each lot is tested for sterility, pH, osmolality, endotoxin level, and functionally tested to support the growth of epithelial cells.
2. Additional information is available upon request.

Limited Use Label License (LULL)

Limited Use Label License: EpiX Technology, covered under US Patent Application No. 15/296,831 and 15/296,960, PCT Application US2016/025396, and additional national phase filings in other jurisdictions.

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