Human Airway Organoid Medium

V03

Catalog No.: O5030
Specification: 100 mL/500 mL
Storage: The aliquoted organoid medium must be stored at -20°C and is valid for 2 years. Avoid repeated freezing and thawing. After thawing, the organoid complete medium can be stored at 4°C and is recommended to be used within two weeks. The organoid medium contains antibacterial and antifungal antibiotics.

Product Introduction:

Human Tracheal Organoid Medium is a chemically defined cell culture medium used to establish and maintain human tracheal organoids derived from human tracheal stem cells. The self-renewal of the airway epithelium is driven by the proliferation of basal stem cells. Human tracheal organoids contain basal cells, ciliated cells, secretory cells, and a small number of neuroendocrine cells. The organoids therefore exhibit all the characteristics of the airway epithelium in terms of architecture, cell-type composition, and self-renewal dynamics, and thus have great potential for airway development and disease research.

Other Required Materials and Reagents:

Matrigel, epithelial organoid basal medium, organoid culture anti-adhesion rinse solution, organoid dissociation solution, tissue dissociation solution, red blood cell lysis buffer, biopsy tissue preservation solution, EDTA (0.5 M, pH 8.0), fetal bovine serum, DPBS (1×), liquid, without calcium and magnesium, cell strainer 100 μm, cell culture plates 96/48/24/12/6 well, centrifuge tubes 1.5/5/15/50 mL, cell culture dishes 6/10 cm.

Operating Procedure: (for reference only)

I. Instructions for Human Tracheal Organoid Complete Medium

1. After receiving the organoid medium, place it in a 4° C refrigerator to thaw.
2. After the medium is completely thawed, invert it several times to mix thoroughly, then aliquot it according to daily requirements in a biological safety cabinet or clean bench. It is recommended to aliquot into 10 mL/tube.
3. Seal the aliquoted medium and store at -20° C. When in use, take out the aliquoted medium and allow it to equilibrate to room temperature before use.

II. Establishment and Subculture of Human Tracheal Organoids

Note: Research involving primary human tissue materials must comply with all relevant institutional and governmental regulations. Informed consent must be obtained from all subjects before collecting primary human tissue materials.

a) Establishment of Primary Human Tracheal Organoids

1. Primary tissue samples should be placed in a sample tube containing pre-cooled biopsy preservation solution (2–8°C) within 5 min of excision. The sample must be completely covered by the biopsy preservation solution (if the sample has been placed in other buffers or media, replace the entire solution with pre-cooled biopsy preservation solution). Transport to the laboratory at low temperature (2–8°C).
2. Before the experiment, place the Matrigel on ice at 4°C to thaw, and at the same time take out the medium to equilibrate to room temperature. Centrifuge tubes, test tubes, or plastic pipette tips that come into contact with cells need to be rinsed with the organoid culture anti-adhesion rinse solution before use.
3. In a clean bench, transfer the sample to a culture dish and assess whether the obtained tissue is composed entirely of epithelium. If fat or muscle tissue is present, use surgical scissors or a scalpel and forceps under a stereomicroscope to remove as much of these non-epithelial components as possible. If there is no fat or muscle tissue, proceed immediately to the next step.
4. Rinse the tissue twice with epithelial organoid basal medium or DPBS containing double antibiotics.
5. Use forceps to transfer the tissue to a 1.5 mL centrifuge tube, and on ice use sterile tissue scissors to cut the tissue into pieces of 0.5–2 mm^2 in size.
6. Resuspend the minced tissue in a volume of tissue dissociation solution equal to 50 times the tissue volume, transfer to a 15 mL centrifuge tube, and pipette to resuspend the tissue pieces. Digest by horizontal shaking on a constant-temperature shaker at 37°C and 100 rpm for 15–30 minutes, observing the tissue digestion every 10 minutes. When the tissue pieces are clearly dispersed and the suspension is turbid, take an appropriate

amount of the tissue digestion suspension for microscopic examination. Terminate digestion when many viable cell clumps are present in the suspension. If the tissue pieces are large or incompletely digested, the digestion time may be appropriately extended. During digestion, pipette tips of different sizes (in order from large to small, e.g., 10 mL, 5 mL, 1 mL) can be used to pipette the tissue digestion suspension to help complete digestion. When most tissue fragments can pass through a 1 mL pipette tip, the digestion process is complete.

7. Add FBS to the tissue digestion mixture to a final concentration of 2%, and filter through a 100 μm cell strainer.

8. Collect the filtrate and centrifuge at 250g for 3 minutes at 4°C. If a visible red pellet is present, aspirate the supernatant, add 2 mL of red blood cell lysis buffer to resuspend the pellet, lyse the red blood cells at room temperature for 1 minute, and centrifuge at 250g for 3 minutes at 4°C.

9. Aspirate the supernatant and resuspend the pellet in epithelial organoid basal medium, centrifuge at 250g for 3 minutes at 4°C, and repeat this step to completely remove the dissociation solution and FBS. Before centrifugation, an appropriate amount of the suspension may be taken for cell viability detection.

10. Aspirate the supernatant, and according to the culture requirements, take an appropriate amount of the cell pellet, add Matrigel (>70%), and mix on ice (note: the mixing and pipetting action should be gentle to avoid generating large numbers of bubbles; mixing at room temperature must be completed within 15 seconds), then place on ice after mixing. Note: The Matrigel should be kept on ice to prevent it from solidifying. Proceed with this process as quickly as possible. Approximately 10,000 cells should be seeded in 25 μL of Matrigel. Do not over-dilute the Matrigel (the Matrigel ratio should be >70% (Matrigel volume/total volume)), as this may inhibit the proper formation of the solid droplet.

11. Pipette the mixed suspension of Matrigel and cells into the center of the bottom of the culture well plate, and use the pipette tip to spread the suspension slightly, taking care to avoid the suspension contacting the side walls of the well plate. (Recommended: seed 3–10 μL/well in a 96-well plate, 10–20 μL/well in a 48-well plate, 20–30 μL/well in a 24-well plate).

Note: Once the organoids are resuspended in Matrigel, proceed with seeding as soon as possible, because the Matrigel may solidify in the test tube or pipette tip.

12. Place the culture plate in an incubator at 37°C and 5% CO_2 for 15–25 minutes to allow the Matrigel to solidify.

13. After the Matrigel is completely solidified, slowly add room-temperature-equilibrated organoid complete medium along the wall to avoid disrupting the solidified structure. (Recommended: add 100 μL/well to a 96-well plate, 250 μL/well to a 48-well plate, 500 μL/well to a 24-well plate). Note: Do not add the medium directly on top of the Matrigel droplet, as this may disrupt the solidified structure.

14. Place the culture plate in a constant-temperature incubator at 37°C and 5% CO_2.

15. Change the medium every 2–3 days, carefully aspirating the medium from the wells and adding fresh room-temperature-equilibrated organoid complete medium.

16. Closely monitor the growth state of the organoids. Ideally, human airway organoids should be established within 7–14 days.

b) Subculture of Human Tracheal Organoids

1. When the organoids have grown to a diameter of 100–500 μm (or have turned black and no longer increase in size), the organoids can be passaged (approximately every 1–2 weeks per passage). For the following operations, all consumables in contact with cells must be rinsed with the rinse solution before use:

2. Aspirate the old medium, add an equal volume of epithelial organoid basal medium, and use a cell scraper or the tip of a pipette to gently scrape off (or pipette) the mixture of Matrigel and organoids. Transfer to a 1.5 mL centrifuge tube (collect at most 2–3 wells of organoids per tube to avoid a volume that is too large and results in low digestion efficiency), pipette 5–10 times to separate the organoids from the Matrigel, and centrifuge at 300g for 3 minutes.

3. Remove the supernatant, add a volume of organoid dissociation solution equal to 5 times the volume of the Matrigel-organoid mixture, pipette to mix, and digest in a 37°C incubator for 5–10 minutes. Take out, pipette to mix, and take 10 μL of the mixture for microscopic examination to check whether it has digested into small cell clumps. If digestion is insufficient, the digestion time may be appropriately extended. If cell counting is required, the cells must be digested into single cells; the digestion time may be extended to 10–20 minutes, then terminate digestion and perform trypan blue staining for cell counting. Closely monitor the digestion process to keep the incubation time in the organoid dissociation solution as short as possible.

4. After digestion is complete, add 5 volumes of epithelial organoid basal medium and pipette to mix to terminate the digestion reaction, then centrifuge at 250g for 3 minutes.

5. Aspirate the supernatant, and wash twice with epithelial organoid basal medium to remove residual dissociation solution. For the last wash, the organoids can be pooled and collected in the same centrifuge tube.

Tel: 86-010-50973105 Web: solarbio.com E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

6. After washing, passage and culture the cells in the centrifuged pellet. Add Matrigel (>70%) to the cell pellet and mix on ice (note: the mixing and pipetting action should be gentle to avoid generating large numbers of bubbles; mixing at room temperature must be completed within 15 seconds), then place on ice after mixing. Note: The Matrigel dilution ratio should be above 70% to ensure the structural stability of the Matrigel during culture.

7. Pipette the mixed suspension of Matrigel and cells into the center of the bottom of the culture well plate, and use the pipette tip to spread the suspension slightly, taking care to avoid the suspension contacting the side walls of the well plate. (Recommended: seed 3–10 μL/well in a 96-well plate, 10–20 μL/well in a 48-well plate, 20–30 μL/well in a 24-well plate). Note: To prevent the Matrigel from solidifying at room temperature, this step should be completed as quickly as possible.

8. Place the culture plate in an incubator at 37°C and 5% CO_2 for 15–25 minutes to allow the Matrigel to solidify.

9. After the Matrigel is completely solidified, slowly add room-temperature-equilibrated organoid complete medium along the wall to avoid disrupting the solidified structure. (Recommended: add 100 μL/well to a 96-well plate, 250 μL/well to a 48-well plate, 500 μL/well to a 24-well plate). Note: Do not add the medium directly on top of the Matrigel droplet, as this may disrupt the solidified structure.

10. Place the culture plate in a constant-temperature incubator at 37°C and 5% CO_2.

11. Change the medium every 2–3 days, carefully aspirating the medium from the wells and adding fresh room-temperature-equilibrated organoid complete medium.

12. Closely monitor the growth state of the organoids until the organoids need to be used for the next experiment.

Precautions:

1. This product is for scientific research use only. Do not use it for medicine, clinical diagnosis or treatment, food, or cosmetics. Do not store it in ordinary residential areas.

2. Product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.

3. For your safety and health, wear a laboratory coat and disposable gloves and a mask during operation.