Human Colonic Organoid Medium, Expansion

V03

Catalog No.: O5000
Specification: 10 mL/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.

Product Introduction:

Human Colon Organoid Medium (Expansion) is a complete medium used to expand human colon organoids. Human colon organoids in the expansion phase are mainly composed of colon stem cells and colon progenitor cells. In terms of self-renewal and differentiation ability, tissue architecture, cell types, and function, colon organoids recapitulate the characteristics of the colonic epithelium in vivo, and are therefore an ideal in vitro model for human colon research.

Operating Procedure: (for reference only)

➢ Instructions for Human Colon Organoid Medium (Expansion)

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.

➢ Establishment and Subculture of Human Colon 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 Colon Organoids

1. Primary tissue samples should be placed in a sample tube containing pre-cooled biopsy preservation solution (placed at 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. Transfer the tissue sample to a culture dish containing pre-cooled DPBS with double antibiotics,

with the intestinal lumen side facing up. Hold one end of the intestinal tissue with surgical forceps using one hand, and gently scrape off the mucus or residual feces on the surface of the intestinal lumen using a surgical blade with the other hand. Then place the colon tissue in a new culture dish containing DPBS with double antibiotics and rinse 2 to 3 times. Cut the rinsed intestinal tissue into pieces of approximately 2 mm × 5 mm, then transfer to a 50 mL centrifuge tube, vortex vigorously for 30 s (vortex vertically up and down 20 times, one up-and-down counts as once, approximately three times per second), discard the supernatant, and repeat 3 times.

5. Digestion: Add 300 μL of 0.5 M EDTA to 30 mL of DPBS solution, to a final EDTA concentration of 5 mM. Place on a shaker at 4°C at 80 rpm and digest for 20–30 min (depending on the sample amount; at 20 min of digestion, the tissue can be pipetted and mixed, then observed under the microscope; if intact crypt structures are observed, proceed to the next step).
6. Washing: After digestion is complete, allow the intestinal segments to settle, and discard the supernatant. Add pre-cooled DPBS, gently mix, allow to settle, and discard the supernatant. Repeat twice to remove the EDTA.
7. Resuspension: Add 30 mL of pre-cooled DPBS containing 0.1% BSA, vortex for 10 s, and filter the supernatant through a 100 μm mesh. Collect the tissue suspension that passes through the mesh, and repeat the collection twice.
8. Collection: Centrifuge at 300g for 3 min at 4°C.
9. Aspirate the supernatant and resuspend the pellet in epithelial organoid basal medium, and take the suspension for crypt counting.
10. Centrifuge at 300g for 3 min at 4°C. 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 50 crypts 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.

B. Subculture of Human Colon Organoids

1. When the organoids have grown to a diameter of 600 μm (or have turned black and no longer increase in size), the organoids can be passaged (approximately every 1 week 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 3 minutes. Take out, pipette to mix, and take 10 μL of the mixture for microscopic examination to check whether it has digested into organoid clumps of approximately 300 μm.

[Note] In the following steps, the pipetting force and number of times for resuspending the pellet need to be controlled, so that the organoid clumps are controlled at a size of approximately 300 μm.

4. After digestion is complete, add 5 volumes of epithelial organoid basal medium, gently 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.
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). Approximately 50 organoid clumps should be seeded in 25 μL of Matrigel. 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℃ 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. The organoid medium contains antibacterial and antifungal antibiotics.
2. 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.
3. Product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
4. For your safety and health, wear a laboratory coat and disposable gloves and a mask during operation.