Mouse Colonic Organoid Growth Medium

Introduction

This medium is a chemically defined, serum-free culture medium for the establishment and maintenance of mouse colon organoids. The organoids cultured with this medium exhibit colon epithelium-like structures, making them ideal models for studying colon physiology and pathology. Additionally, this product is a ready-to-use medium requiring no additional factors, which is more convenient for use.

Components and Storage

Components \ Size	100 mL	500 mL	Storage
Mouse Colonic Organoid Growth Medium	100 mL	500 mL	-20°C
Shipping: Dry ice	Shelf life: 6 months		

Materials Required but Not Included

Products	Catalog number
Organoid Wash Buffer	K2846
Gastrointestinal Tissue Dissociation Solution	K2848
GFR Basement Membrane Matrix (Phenol Red-Free)	EM1001
Organoid Dissociation Solution	K2843
Organoid Cryopreservation Medium	K2844
100 μm cell strainer	-
24-well cell culture plate	-
15, 50 mL centrifuge tubes	-
1.5 mL EP tubes	-

Protocol

1. **Preparation before use:**

    1. Thaw GFR Basement Membrane Matrix (Phenol Red-Free) at 4°C overnight in advance.
    2. Thaw components stored at -20°C in advance, and aliquot the Mouse Colonic Organoid Growth Medium to avoid repeated freeze-thaw cycles.

2. Construction of colonic organoids from primary tissues

1. Isolate colon

    1) Sacrifice mice in accordance with animal ethics regulations. Harvest a certain length of colon and remove any membrane, fat and blood vessels. Plate the colonic segment in a 10 cm dish containing 6 mL of Organoid Wash Buffer. Use forceps to squeeze out the colonic contents, or flush the colon using a pipette to inject the Organoid Wash Buffer from one of the open end of the colon. Then cut colon open lengthwise with surgical scissors.

***Note:** If the experiment is not possible immediately after isolation, store the colon in the Tissue Storage Solution (K2842). For detailed protocol, please refer to the manual of Tissue Storage Solution.

    2) Place the segments in a new dish, add 6 mL of Organoid Wash Buffer, spread out the colon lining with forceps or a glass slide, and gently scrape off the villi on the inner wall.

    3) Transfer the colon to a new dish containing 6 mL of Organoid Wash Buffer, and cut the colon into 5 mm pieces with surgical scissors.

2. Raw tissue analysis (optional)

    1) If additional immunoassays are required, take 1-2 tissue blocks and fix with a fixative solution (4% PFA or 10% formalin).

    2) For molecular analysis (whole exome/genome/mRNA sequencing) or biochemical analysis (WB or proteomics), soak 1-2 tissue blocks in RNAlater™ and freeze them at -80°C.

3. Obtain colonic pieces

    1) Transfer the pieces to a 1.5 mL EP tube and further cut it into 2-4 mm pieces with surgical scissors.

    2) Transfer the pieces to a 15 mL centrifuge tube.

    3) Add 5 mL of Organoid Wash Buffer and subsequently vortex the tube for 30 s.

    4) Let the colonic pieces settle by gravity for 10 s, and carefully remove the supernatant.

    5) Repeat steps 3-4 for a total of 3-5 wash of colonic pieces.

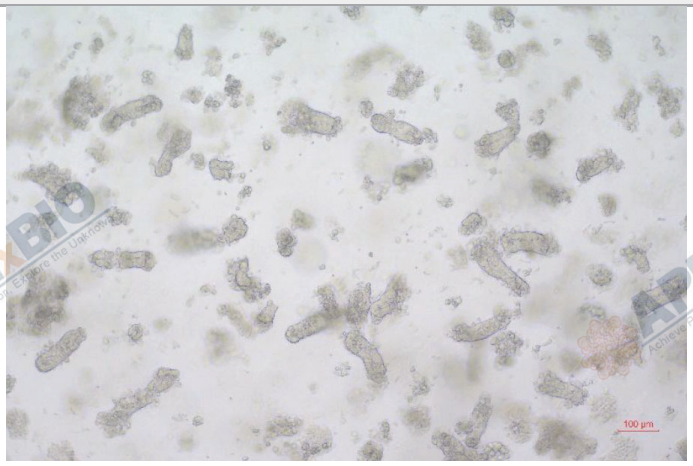
4. Tissue digestion

    1) Add 6 mL of Gastrointestinal Tissue Dissociation Solution, seal the tube with parafilm, and place at 4°C to digest.

    2) Every 5 min, vortex for 30 s, and aspirate 30 µL of suspension and observe under the microscope.

    3) The digestion time should be controlled within 30 min, and the digestion can be stopped when observing 10-100 crypts around 100 µm in diameter under the microscope (as shown in the figure below).

***Note:** Over-digestion (e.g., single cell) can affect subsequent organoid construction and reduce viability.



4) Filter the suspension by a 100 μm cell strainer and supplement with Organoid Wash Buffer to 26 mL.

5) Evenly divide the filtered suspension into two 15 mL centrifuge tubes and then centrifuged at 300 g or 1500 rpm for 5 min. Discard the supernatant.

6) Add 10 mL of Organoid Wash Buffer. Centrifuge at 300 g or 1500 rpm for 5 min and discard the supernatant.

***Note:** After centrifugation, remove the supernatant as much as possible. Because the concentration of Matrigel needs to be above 70% to make structural stability.

5. Organoid culture

1) Mix the pellet and GFR Basement Membrane Matrix (Phenol Red-Free) on ice at a ratio of 1:10. Thaw GFR Basement Membrane Matrix (Phenol Red-Free) at 4°C overnight in advance.

***Note:** If air bubbles appear during the blowing process, absorb the Matrigel below the bubbles, and then vigorously tap the table with the tube to break the bubbles.

2) Using a 24-well plate as an example, drop the Matrigel/Cell mixture evenly in the center of the well, 20-60 μL per drop.

3) Place the plate in a 37°C incubator to set the Matrigel domes.

4) After 15 min, add 500 μL of room temperature Mouse Colonic Organoid Growth Medium to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes directly.

***Note:** Equilibrate Mouse Colonic Organoid Growth Medium at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% CO_2 incubator. Exchange Mouse Colonic Organoid Growth Medium every 1-2 days.

***Note:**

a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.

b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells

and replace with Mouse Colonic Organoid Growth Medium after 4 h.

3. Organoid passage

1. Organoid harvest

1) Discard the medium and slowly add pre-chilled Organoid Wash Buffer to the wells (PBS containing 1% antibiotics can also be used).

2) Pipet Matrigel/Organoid/Medium mixture up and down to release organoid from the Matrigel.

***Note:** If finding that organoids are attached to the wall, scrape the organoid off with a pipette tip.

3) Transfer the mixture to a new 15 mL tube and add Organoid Wash Buffer to make up the volume to 12 mL.

4) Place the tubes in -20°C for 6-12 min or 4°C for 30 min.

***Note:** Brief periods of low temperature do not affect the activity of organoids, and Matrigel can also be removed by taking advantage of the properties of Matrigel dissolving at low temperatures.

5) Centrifuge at 300 g or 1500 rpm for 5 min, and discard the supernatant.

2. Organoid dissociation

Organoids are generally passaged through a combination of mechanical and enzymatic dissociation. Some organoids may only require mechanical dissociation, while others may only require enzymatic dissociation. Some may require both methods. Not to reduce organoids to single cells during passaging.

Mechanical dissociation method

1) Suspend pellet in 1-2 mL of Organoid Wash Buffer. Pipet the suspension up and down.

2) When observing the organoids reach 100-300 μm in diameter, stop the digestion. The bud structure of the colonic organoid should be pipetted down intact.

3) Add Organoid Wash Buffer to a total volume of 12 mL.

4) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

Enzymatic dissociation method

1) Suspend pellet in 1-2 mL of room temperature Organoid Dissociation Solution. Pipet the suspension up and down.

***Note:** Equilibrate Organoid Dissociation Solution at room temperature in advance.

2) Every 2 min, observe the size of organoids. When the organoids reach 100-200 μm in diameter, stop the digestion. It is recommended that the digestion time should not exceed 6 minutes.

3) Add Organoid Wash Buffer to 12 mL.

4) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

3. Organoid passage

1) Mix the pellet and GFR Basement Membrane Matrix (Phenol Red-Free) on ice at a ratio of 1:15. Thaw GFR Basement Membrane Matrix (Phenol Red-Free) at 4°C overnight in advance.

***Note:** If air bubbles appear during the blowing process, absorb the Matrigel below the bubbles, and then vigorously tap the table with the tube to break the bubbles.

2) Using a 24-well plate as an example, drop the Matrigel/Organoids mixture evenly in the center of the well, 20-60 μL per drop.

3) Place the plate in a 37°C incubator to set the Matrigel domes.

4) After 15 min, add 500 μL of room temperature Mouse Colonic Organoid Growth Medium to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes directly.

***Note:** Equilibrate Mouse Colonic Organoid Growth Medium at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% CO_2 incubator. Exchange Mouse Colonic Organoid Growth Medium every 2-4 days.

***Note:**

a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.

b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells and replace with Mouse Colonic Organoid Growth Medium after 4 h.

4. Organoid cryopreservation

Cryopreservation must be performed when the organoids are in optimal condition. Fast-growing organoids (up to 300-500 μm in diameter) can be cryopreserved when they reach 150-200 μm in diameter. Slow-growing organoids (up to 100-300 μm in diameter) can be cryopreserved when they reach 100-150 μm in diameter.

During the cryopreservation, gently pipetting to ensure the integrity of the organoids.

1. Organoid harvest

1) Discard the medium and slowly add pre-chilled Organoid Wash Buffer to the wells (PBS containing 1% antibiotics can also be used).

1) Pipet Matrigel/Organoid/Medium mixture up and down to release organoid from the Matrigel.

***Note:** If finding that organoids are attached to the wall, scrape the organoid off with a pipette tip.

2) Transfer the mixture to a new 15 mL tube and add Organoid Wash Buffer to make up the volume to 12 mL.

3) Place the tubes in -20°C for 6 min or 4°C for 30 min.

***Note:** Brief periods of low temperature do not affect the activity of organoids, and Matrigel can also be removed by taking advantage of the properties of Matrigel dissolving at low temperatures.

4) Centrifuge at 300 g or 1500 rpm for 5 min, and discard the supernatant.

2. Organoid cryopreservation

1) Depending on the pellet volume, mix the pellet and Organoid Cryopreservation Medium at a ratio of 1:10.

2) After mixing, aliquot the organoids into cryopreservation vials, and perform cryopreservation by programmed cooling.

***Note:** Cryopreserved organoids can be stored at -80°C for one month, and for long-term storage, store the vials in a liquid nitrogen tank.

3. Organoid resuscitation

1) Equilibrium Organoid Wash Buffer to room temperature in advance. Add 2 mL of room temperature Organoid Wash Buffer in a 15 mL centrifuge tube. 2% serum can be added to the Organoid Wash Buffer.

2) Remove the vials from the liquid nitrogen tank and thaw them in a 37°C water bath for 1-2 min.

3) Slowly transfer the thawed organoid suspension to the 15 mL centrifuge tube containing Organoid Wash Buffer. Add another 8 mL of Organoid Wash Buffer.

4) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

5) Add 2 mL of room temperature Organoid Wash Buffer (without serum) and gently resuspend the organoids.

6) Add 8 mL of Organoid Wash Buffer.

7) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

4. Organoid culture

1) Mix the pellet and GFR Basement Membrane Matrix (Phenol Red-Free) on ice at a ratio of 1:10. Thaw GFR Basement Membrane Matrix (Phenol Red-Free) at 4°C overnight in advance.

***Note:** If air bubbles appear during the blowing process, absorb the Matrigel below the bubbles, and then vigorously tap the table with the tube to break the bubbles.

2) Using a 24-well plate as an example, drop the Matrigel-Cell mixture evenly in the center of the well, 20-60 μL per drop.

3) Place the plate in a 37°C incubator to set the Matrigel domes.

4) After 15 min, add 500 μL of room temperature Mouse Colonic Organoid Growth Medium to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes

directly.

***Note:** Equilibrate Mouse Colonic Organoid Growth Medium at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% CO_2 incubator. Exchange Mouse Colonic Organoid Growth Medium every 2-4 days.

***Note:**

a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.

b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells and replace with Mouse Colonic Organoid Growth Medium after 4 h.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com