Mouse Intestinal Organoid Kit

Introduction

This kit is a comprehensive system for the establishment and maintenance of mouse small intestinal organoids. This kit contains a complete set of reagents for tissue preservation, organoid construction, organoid passage, and cryopreservation. This kit is ideal for organoid culture and does not require additional reagents other than Matrigel. Organoids cultured with this kit have a small intestinal epithelium-like structure and are ideal models for small intestinal physiology and pathology studies.

Components and Storage

Components \ Size	3-5 Assays	Storage
Mouse Intestinal Organoid Growth Medium	100 mL	-20°C
Gastrointestinal Tissue Dissociation Solution	50 mL	4°C
Organoid Dissociation Solution	40 mL	-20°C
Organoid Cryopreservation Medium	40 mL	4°C
Tissue Storage Solution	100 mL	-20°C
Organoid Wash Buffer	500 mL	-20°C
Shipping: Dry ice	Shelf life: 6 months	

Materials Required but Not Included

Products	Catalog number
GFR Basement Membrane Matrix (Phenol Red-Free)	EM1001
100 μm cell strainer	-
24-well cell culture plate	-
15 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 Intestinal Organoid Growth Medium to avoid repeated freeze-thaw cycles.

2. Construction of small intestinal organoids from primary tissues

1. Isolate small intestine

   1) Sacrifice mice in accordance with animal ethics regulations. Harvest a certain length of small intestine (e.g., 20 cm) and remove any membrane, fat and blood vessels. Plate the intestinal segment in a 10 cm dish containing 6 mL of Organoid Wash Buffer. Use forceps to squeeze out the intestinal contents, or flush the intestine using a pipette to inject the Organoid Wash Buffer from one of the open end of the intestine. Then cut intestinal open lengthwise with surgical scissors.

***Note:** If the experiment is not possible immediately after isolation, store the intestine 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 intestinal lining with forceps or a glass slide, and gently scrape off the villi on the inner wall.

   3) Transfer the intestine segments to a new dish containing 6 mL of Organoid Wash Buffer, and cut the small intestine into 50 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 intestinal pieces

   1) Transfer the intestine pieces to a 1.5 mL EP tube and further cut it into 10-20 mm intestinal pieces with surgical scissors.

   2) Transfer the intestinal 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 intestinal pieces settle by gravity for 10 s, and carefully remove the supernatant.

   5) Repeat steps 3-4 for a total of 5-10 wash of intestinal pieces.

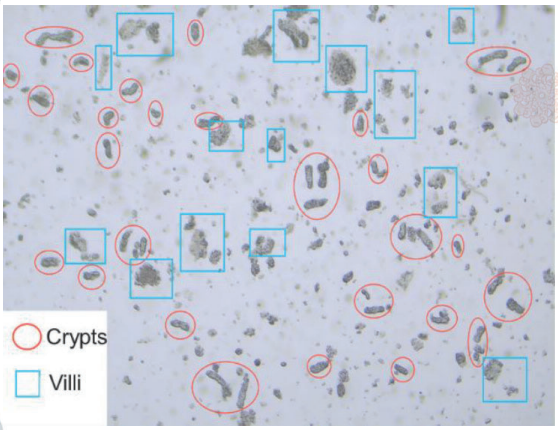
4. Tissue digestion

   1) Add 6 mL of Gastrointestinal Tissue Dissociation Solution, seal the tube with parafilm, and place in 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. At the same time, significant cavities can be observed after the crypts in the intestinal wall have fallen off.



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) If observing a red precipitate, add 2 mL of Red Blood Cell Lysis Buffer (K1169) to resuspend the precipitate, and then let it stand at room temperature for 3 min.

***Note:** If the tissue has been harvested for more than 48 h, it is not recommended to use Red Blood Cell Lysis Buffer.

7) 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 small intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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 Intestinal 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