# Mouse Liver Ductal Organoid Kit (Differentiation)

## Introduction

This kit is a comprehensive system for the differentiation of mouse liver ductal 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. Using this kit, Liver Ductal organoids can differentiate into liver cells, which are ideal models for liver physiology and pathology studies.

## Components and Storage

| Components \ Size | 3-5 Assays | Storage |
|---|---|---|
| Mouse Liver Ductal Organoid Growth Medium (Expansion) | 50 mL | -20°C |
| Mouse Liver Ductal Organoid Growth Medium (Differentiation) | 50 mL | -20°C |
| Tissue Dissociation Solution | 50 mL | -20°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 Expansion Medium and Differentiation Medium to avoid repeated freeze-thaw cycles.

## 2. Construction of organoids from primary tissues

1. Liver isolation

    1) Sacrifice mice in accordance with animal ethics regulations. Harvest intact liver tissue and place it in a dish. Wash the liver with Organoid Wash Buffer for 2-3 times.

***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) Cut the liver tissue into the size of a grain of rice (2-4 $mm^3$) with surgical scissors.

    3) Add 5-10 mL of Organoid Wash Buffer to repeatedly pipette and wash.

    4) Remove the Organoid Wash Buffer.

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

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. Tissue cutting

    1) Transfer tissue to a 1.5 mL EP tube and further cut them with surgical scissors.

    2) Transfer tissue to a 15 mL centrifuge tube.

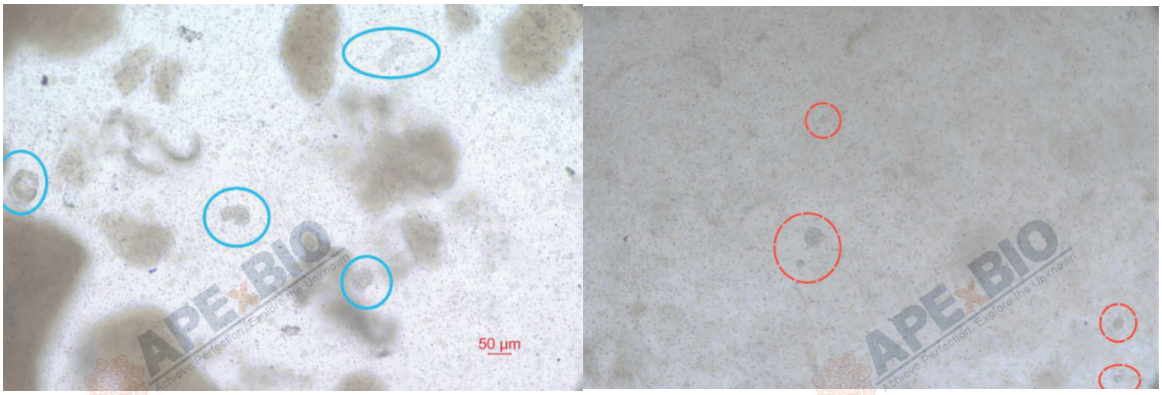
4. Tissue digestion

    1) Add 6 mL of Tissue Dissociation Solution, seal the tube with parafilm, and place in 37°C bath 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 60 min, and the digestion can be stopped when observing 3-10 cell cluster 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) 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 Liver Ductal Organoid Growth Medium (Expansion) to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes directly.

***Note:** Equilibrate Mouse Liver Ductal Organoid Growth Medium (Expansion) at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% $CO_2$ incubator. Exchange Mouse Liver Ductal Organoid Growth Medium (Expansion) 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 Liver Ductal Organoid Growth Medium (Expansion) 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 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 40-200 μm in diameter, stop the digestion.

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 40-200 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 Liver Ductal Organoid Growth Medium (Expansion) to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes directly.

***Note:** Equilibrate Mouse Liver Ductal Organoid Growth Medium (Expansion) at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% $CO_2$ incubator. Exchange Mouse Liver Ductal Organoid Growth Medium (Expansion) 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 Liver Ductal Organoid Growth Medium (Expansion) after 4 h.

## 4. Organoid differentiation

**Primary tissues cannot directly construct mouse hepatocyte organoids. First establishment Liver Ductal organoids, and then induce the Liver Ductal organoids with differentiation medium.**

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 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

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

2) When observing the organoids reach 40-200 μm in diameter, stop the digestion.

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.

3. Organoid expansion

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 Liver Ductal Organoid Growth Medium (Expansion) to each well by pipetting the medium gently down the sidewall. Do not pipette the medium onto the domes directly.

***Note:** Equilibrate Mouse Liver Ductal Organoid Growth Medium (Expansion) at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% $CO_2$ incubator. Exchange Mouse Liver Ductal Organoid Growth Medium (Expansion) 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 Liver Ductal Organoid Growth Medium (Expansion) after 4 h.

4. Organoid differentiation

1) When the organoids reach 200 μm in diameter, remove the medium.

2) Add 700-1000 μL Mouse Liver Ductal Organoid Growth Medium (Differentiation) per well to induce differentiation.

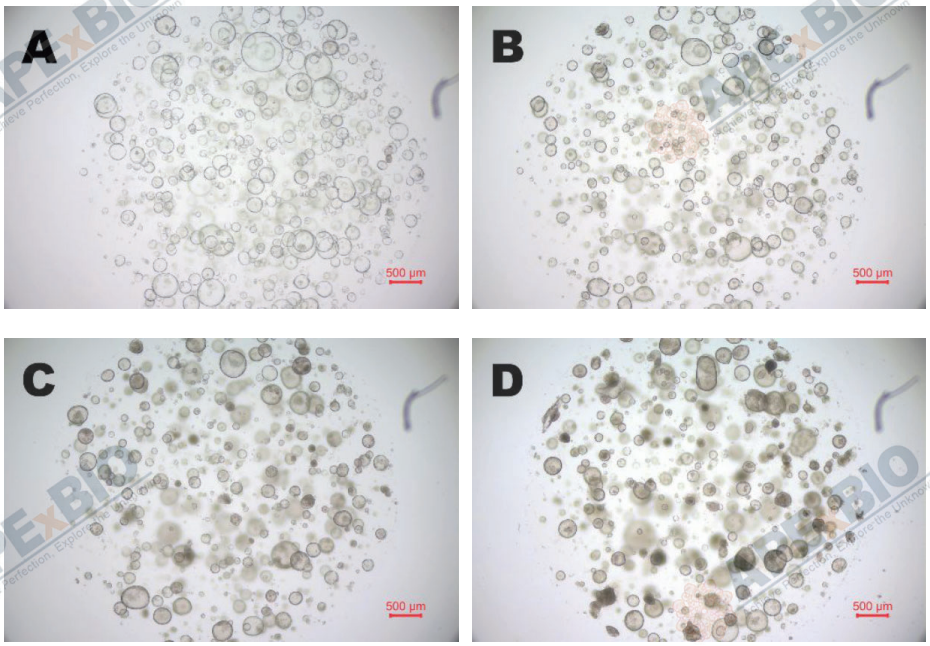
3) Continue to culture for 7-14 days, differentiation is completed.

***Note:** Differentiated organoids cannot be passaged. To obtain more liver cell organoids, more liver ductal organoids need to be

differentiated.

4) During the differentiation, if organoids diameter > 400 μm, perform passage steps to control their size. Take care to add the differentiation medium at the end rather than the expansion medium.

5) The whole process of differentiation can be referred to the figure below.




Organoids at (A) day 0, (B) Day 2, (C) Day 4, and (D) Day 8.

## 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.

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