Mouse Liver Ductal Organoid Growth Medium (Differentiation)

Introduction

This medium is a chemically defined, serum-free culture medium for the differentiation of mouse liver ductal organoids. The organoids induced by this medium can differentiate into hepatocyte-like cells, making them ideal models for studying liver 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 Liver Ductal Organoid Growth Medium (Differentiation)	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
GFR Basement Membrane Matrix (Phenol Red-Free)	EM1001
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 Differentiation Medium to avoid repeated freeze-thaw cycles.
2. **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.

For protocol of Liver Ductal organoids construction, please refer to the CM1035 or K2935 manual. Here is differentiation protocol after the construction of Liver Ductal 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).

    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.

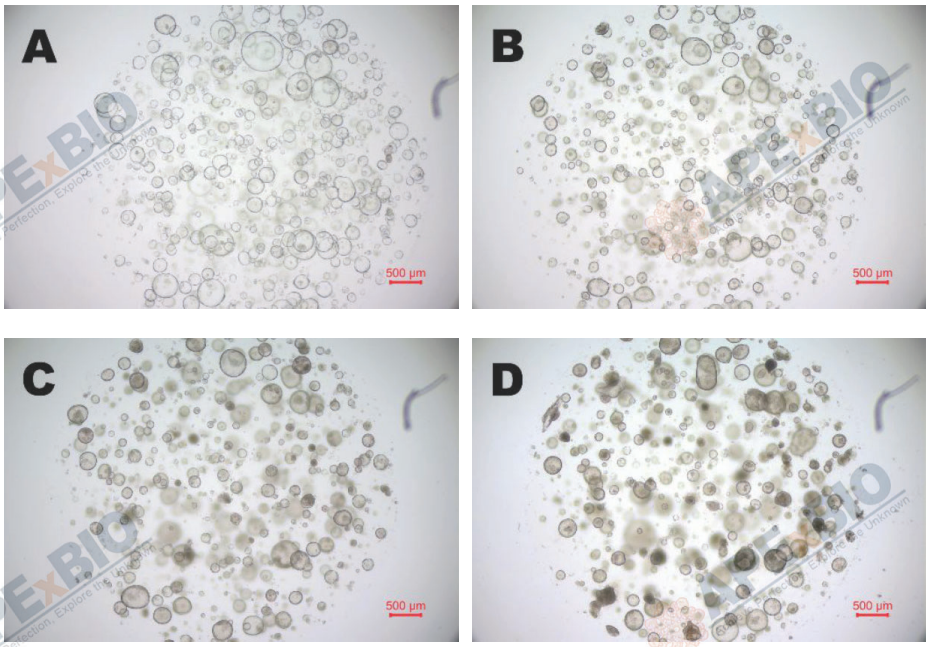
2) Add 700-1000 μL Mouse Liver Ductal Organoid Growth Medium (Differentiation) per well to induce differentiation.

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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