Human Hepatocyte Organoid Kit

Introduction

This kit is a comprehensive system for human hepatocyte organoid culture. 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 exhibit a hepatocyte-like structure, making them ideal models for studying liver physiology and pathology.

Components and Storage

Components \ Size	5 Assays	Storage
Human Liver Ductal Organoid Growth Medium (Expansion)	50 mL	-20°C
Human 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 Human Liver Ductal Organoid Growth Medium (Expansion) to avoid repeated freeze-thaw cycles.

2. Construction of organoids from primary tissues

1. Tissue isolation

   1) Isolate tissue in accordance with local ethics regulations. Under sterile conditions, isolate tissue, wash tissue with saline several times, and then place in a conical tube containing Tissue Storage Solution. Keep tissue at 4°C until start the experiment.

***Note:** For detailed protocol, please refer to the manual of Tissue Storage Solution (K2842).

   2) Transfer the pieces to a dish and further cut it into 2-4 mm^3 pieces with surgical scissors.

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

   4) Remove the Organoid Wash Buffer.

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

   6) At this point, 1–2 tissue pieces can be collected for in situ analysis.

2. Tissue digestion

   1) Transfer the pieces to a 1.5 mL centrifuge tube and further cut them into 0.5 mm^2 pieces with surgical scissors.

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

   3) Add 6 mL of Tissue Dissociation Solution, seal the tube with parafilm, and place in 37°C bath to digest.

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

   5) The digestion time should be controlled within 40 min, and the digestion can be stopped when observing 10-100 cell clumps around 100 μm in diameter under the microscope.

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

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

***Note:** For biopsy samples, to reduce losses, add the Organoid Wash Buffer directly after digestion without filtration.

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

   8) 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.

   9) 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.

3. 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 Human 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 Human 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 Human 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 Human 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 Human 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 Human 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 Human 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 Human Liver Ductal Organoid Growth Medium (Expansion) after 4 h.

4. Organoid differentiation

Primary tissues cannot directly construct 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 Human 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 Human 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 Human 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 Human Liver Ductal Organoid Growth Medium (Expansion) after 4 h.

4. Organoid differentiation

1) When the organoids reach 100-200 μm in diameter, remove the medium.

2) Add 700-1000 μL Human 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.

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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