Human Hepatocellular Cancer Organoid Growth Medium

Introduction

Patient-derived tumor organoids can retain the genetic characteristics of the primary tumor, which have a wide range of applications in disease modeling, drug screening, and exploration of tumor development mechanisms. This medium is a chemically defined, serum-free culture medium for the establishment and maintenance of human hepatocellular cancer organoids. 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
Human Hepatocellular Cancer 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
Tissue Dissociation Solution	K2841
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 Human Hepatocellular Cancer Organoid

Growth Medium 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 tumor 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) Cut the 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 into pieces with surgical scissors.

   2) Transfer pieces 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 40 min, and the digestion can be stopped when observing 10-100 cell cluster around 100 μm in diameter under the microscope.

***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.

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

   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 Human Hepatocellular Cancer 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 Human Hepatocellular Cancer Organoid Growth Medium at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% CO_2 incubator. Exchange Human Hepatocellular Cancer 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 Human Hepatocellular Cancer 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 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 (Vacuolar or parenchyma)

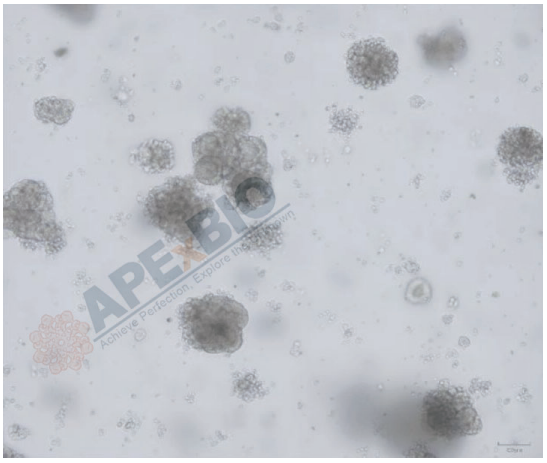
1) Suspend pellet in 1-2 mL of Organoid Wash Buffer. Pipet the suspension up and down.

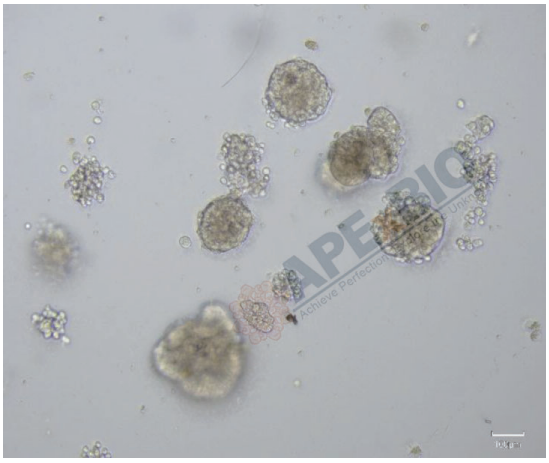
2) When observing the organoids reach 40-200 μm in diameter, stop the digestion.

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 (Sac-like or thick-walled)





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 Hepatocellular Cancer 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 Human Hepatocellular Cancer Organoid Growth Medium at room temperature for 15 min in advance.

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***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.

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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.

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***Note:** Equilibrate Human Hepatocellular Cancer Organoid Growth Medium at room temperature for 15 min in advance.

5) Incubate the plate in a 37°C, 5% CO_2 incubator. Exchange Human Hepatocellular Cancer 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 Human Hepatocellular Cancer 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.

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