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# Human Cholangiocarcinoma Organoid Kit

## 1 Contents

| Cat.No. | Component | HY-K6106-100 mL | HY-K6106-500 mL |
|---|---|---|---|
| HY-K6106-A | Cholangiocarcinoma Organoid Basal Medium A | 100 mL | 500 mL |
| HY-K6106-B | Cholangiocarcinoma Organoid Culture Supplement B (50x) | 1 mL × 2 | 10 mL |
| HY-K6106-C | Cholangiocarcinoma Organoid Culture Supplement C (250x) | 0.4 mL | 1 mL × 2 |

## 2 Introduction

MCE Human Cholangiocarcinoma Organoid Kit contains Cholangiocarcinoma Organoid Basal Medium A, Cholangiocarcinoma Organoid Supplement B (50x), Cholangiocarcinoma Organoid Supplement C (250x). This product can be used to efficiently construct human cholangiocarcinoma organoid. The organoid of cholangiocarcinoma can highly simulate the tumor microenvironment of cholangiocarcinoma, which facilitates the observation of the growth and proliferation of cholangiocarcinoma cells and can be used to track the changes of tumor cells under the action of drugs and screen the efficacy and toxicity of drugs.

## 3 Operation Instructions

### 1. Preparation of complete culture medium for cholangiocarcinoma

Prepare complete medium for cholangiocarcinoma organoid according to the following components, mix thoroughly and set aside on ice.

| Reagents | 10 mL | 20 mL | 20 mL | Final concentration |
|---|---|---|---|---|
| Cholangiocarcinoma Organoid Basal Medium A | 9.76 mL | 19.52 mL | 19.52 mL | 1x |
| Cholangiocarcinoma Organoid Culture Supplement B (50x) | 200 µL | 400 µL | 400 µL | 1x |
| Cholangiocarcinoma Organoid Culture Supplement C (250x) | 40 µL | 80 µL | 80 µL | 1x |

### 2. Extraction of tumor cells from primary tissues

**a.** Soak freshly extracted tumor tissues using pre-cooled primary tissue storage solution and store them temporarily in a 4°C refrigerator.

**b.** Rinse with Cholangiocarcinoma Organoid Basal Medium A or PBS to remove non-epithelial tissue components such as fat or muscle under guaranteed aseptic condition.

**c.** Use sterile scissors to divide the rinsed tumor tissue into the smallest possible pieces (approximately 1-2 mm in diameter) in a cell culture dish. Transfer them to a 15 mL conical tube using a 1 mL pipette tip.

**d.** Add an appropriate amount of tumor tissue digest and tissue debris to a 15 mL conical tube, with the volume of digest not exceeding two-thirds of the volume of the conical tube. Incubate the conical tube on a horizontal shaker at 37°C for 0.5-1 hour until most of the debris can be aspirated by the 1 mL pipette tip.

**e.** Add an appropriate amount of FBS to the tumor tissue digestion mixture at a final concentration of 2% FBS and then filter on ice using a 100 µm cell strainer.

**f.** Centrifuge at 250 g for 3 min at 4°C using a cryogenic centrifuge and collect the cell pellet. If the cell pellet is red, aspirate the supernatant and resuspend with 1-2 mL of Erythrocyte Lysate, minimizing tip contact with the bottom of the tube. After lysis for 1 min at room temperature, centrifuge the cells at 250 g for 3 min at 4°C in a cryocentrifuge and collect the cell pellet.

**g.** Resuspend the collected cell pellet with an appropriate amount of Cholangiocarcinoma Organoid Basal Medium A. Collect the cell pellet by centrifugation at 250 g for 3 min at 4°C in a cryogenic centrifuge. Repeat this step 1-2 times.

### 3. Construction of organoid

**a.** Resuspend the collected tumor cells in MCE Basement Membrane Matrix on ice. The recommended cell density is $4x10^4$/100 µL Basement Membrane Matrix. It is recommended that 100% MCE Basement Membrane Matrix be used to resuspend tumor cells. If dilution is required, please ensure that the ratio of MCE Basement Membrane Matrix volume to the volume of organoid medium used for dilution is greater than 2:1.

**b.** Inject MCE Basement Membrane Matrix along with the cell suspension into the bottom of a 24-well cell culture plate quickly using a pre-wetted 200 µL pipette tip. It is recommended 25-35 µL of suspension per well. Please avoid air bubbles as much as possible. The cell culture plate is then incubated in an incubator at 37°C, 5% $CO_2$ for 15-30 min until gelling.

**c.** Inject 500 µL of cholangiocarcinoma organoid complete medium at the edge of each well slowly to avoid disrupting the existing gel structure after gelling. Then place the cell culture plate back into the incubator at 37°C, 5% $CO_2$.

**d.** Replace 500 µL of pre-warmed cholangiocarcinoma organoid complete medium volume at 37°C every 3-4 days. Cholangiocarcinoma organoid can be observed in 7-10 days.

### 4. Organoid passages

**a.** It is recommended to aspirate the upper medium when the cholangiocarcinoma organoid is larger than 200 µm in diameter or darkened and add 500 µL of Cholangiocarcinoma Organoid Basal Medium A. Use a cell scraper or 1 mL pipette tip to blow to peel the contents of the cell culture wells out of the plate and transfer them to a 1.5 mL EP tube.

**b.** Blow gently until the cholangiocarcinoma organoid is separated from MCE Basement Membrane Matrix using a pipette tip. Then collect the precipitate by centrifugation at 250-300 g for 3 min at room temperature.

**c.** Add 1 mL of Cholangiocarcinoma Organoid Basal Medium A and resuspend and gently blow well until the organoids are dispersed into fragments. If the organoid is difficult to be blown into pieces, use 0.2-0.5 mL of organoid digestion solution in 37°C incubator to digest the organoid until it is dispersed into cell clusters containing 10-50 cells. The digestion time should be limited to 5 min or less. The digestion is then terminated by adding 5 mL Cholangiocarcinoma Organoid Basal Medium A.

**d.** Centrifuge at 250-300 g for 3 min at room temperature. After centrifugation, the supernatant was discarded and washed 1-2 times with Cholangiocarcinoma Organoid Basal Medium A or PBS and then prepared for use.

**e.** Resuspend the collected tumor cells in MCE Basement Membrane Matrix on ice. The recommended cell density is $4x10^4$/100 µL Basement Membrane Matrix. It is recommended that 100% MCE Basement Membrane Matrix be used to resuspend tumor cells. If dilution is required, please ensure that the ratio of MCE Basement Membrane Matrix volume to the volume of organoid medium used for dilution is greater than 2:1.

**f.** Using a pre-wetted 200 µL pipette tip, then quickly inject MCE Basement Membrane Matrix with the cell suspension into the bottom of the 24-well cell culture plate, avoiding air bubbles as much as possible, injecting 25-35 µL of suspension per well. The cell culture plate is then incubated in a 37°C, 5% $CO_2$ incubator for 15-30 min until solidification.

**g.** Inject 500 µL of cholangiocarcinoma organoid complete medium at the edge of each well slowly to avoid disrupting the existing gel structure after gelling. Then place the cell culture plate back into the incubator at 37°C, 5% $CO_2$.

**h.** Replace 500 µL of pre-warmed cholangiocarcinoma organoid complete medium volume at 37°C every 3-4 days.

## 4 Storage

| | | |
|---|---|---|
| Individual Components | Cholangiocarcinoma Basal Medium A | 4°C, 1 year. |
| | Cholangiocarcinoma Supplement B (50x) | -20°C, 1 year. Avoid repeated freeze/thaw cycles. |
| | Cholangiocarcinoma Supplement C (250x) | -20°C, 1 year. Avoid repeated freeze/thaw cycles. |
| Complete Culture Medium | Cholangiocarcinoma Complete Culture Medium | 4°C, 2 weeks or -20°C, 3 months.<br>Avoid repeated freeze/thaw cycles. |

Note: It is recommended that individual components be formulated for use immediately after thawing. It is better to prepare complete culture medium fresh before the experiment, otherwise please make aliquots for freezing.

## 5 Precautions

1. Tumor cells need to be kept sterile when extracted from primary tumor tissue to avoid contamination from subsequent experiments.
2. Observe the fragmentation status of the organoid during passaged digestion, and terminate the digestion when small cell clusters (10-50 cells) appear to avoid prolonging the subsequent growth viability of the organoid.
3. Operations involving MCE Basement Membrane Matrix need to be kept at low temperature throughout. MCE Basement Membrane Matrix should be injected rapidly into the cell culture wells after resuspension with the cells, while avoiding air bubbles.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.