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Mouse Liver Ductal Organoid (expansion) Kit

1 Contents

Cat.No.	Component	HY-K6118-100 mL	HY-K6118-500 mL
HY-K6118-A	Mouse Liver Ductal Organoid (expansion) Basal Medium A	100 mL	500 mL
HY-K6118-B	Mouse Liver Ductal Organoid (expansion) Supplement B (50x)	1 mL × 2	10 mL
HY-K6118-C	Mouse Liver Ductal Organoid (expansion) Supplement C (250x)	0.4 mL	1 mL × 2

2 Introduction

MCE Mouse Liver Ductal Organoid Kit contains Liver Ductal Organoid Basal Medium A, Liver Ductal Organoid Supplement B (50x), Liver Ductal Organoid Supplement C (250x). This product can be used to efficiently construct mouse liver ductal organoid.

Liver ductal organoids can be used for various applications such as disease modelling, organ development, drug screening, personalised medicine, toxicology, and have the potential for applications in regenerative biology.

3 Operation Instructions

1. Preparation of expansion medium for liver ductal

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

Reagents	10 mL	20 mL	50 mL	Final concentration
Mouse Liver Ductal Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Mouse Liver Ductal Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Mouse Liver Ductal Organoid Supplement C (250x)	40 µL	80 µL	200 µL	1x

2. Extraction of cells from primary tissues

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

b. Rinse with Liver Ductal 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 primary tissue into the smallest possible pieces (approximately 5 mm in diameter) in a cell culture dish, ensuring they are small enough to pass through the tip of a 10 mL pipette. Transfer them to a 15 mL conical tube containing 10 mL of cold Liver Ductal Organoid Basal Medium A with 1% FBS.

d. Prepare Organoid Basal Medium A with 10% FBS (10% FBS medium). Coat the inner surface of pipette tips with 10% FBS medium before use to avoid the adherence of the samples on the pipette wall.

e. Wash the samples by pipetting with a 10 mL pipette at least ten times. Stand the tube and let the samples settle by gravity.

f. Aspirate the supernatant with a 10 mL pipette. Add an appropriate amount of tissue digest, preferably not exceeding two-thirds of the volume of the conical tube. Incubate the conical tube on a horizontal shaker at 37°C for no more than 30 minutes.

g. Check digestion under the microscope if the duct structure appears during digestion, and and once it appears, stop digestion by adding FBS to a final concentration of 2% and pipetting gently up and down.

h. Stand the tube and let the samples settle by gravity for 1-2 min. Collect the supernatant into a new tube.

i. Add 10mL of Liver Ductal Organoid Basal Medium A. Stand the tube and let the samples settle by gravity for 1-2 min, and collect the supernatant into a new tube. Collect the cell precipitate after centrifugation at 300 g for 3 min at 4°C using a cryogenic centrifuge.

j. Resuspend the collected cell precipitate by adding 10 mL of Liver Ductal Organoid Basal Medium A, and then collect the cell precipitate by centrifugation at 300 g for 3 min in a low-temperature centrifuge at 4°C. Repeat this step twice.

3. Construction of organoid

a. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. Approximately 40-200 ducts should be plated in 50 µL Basement Membrane Matrix. The volume of Basement Membrane Matrix should be greater than 2/3 of the total diluted volume.

b. 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 gelling.

c. Inject 500 µL of liver ductal organoid expansion 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 liver ductal organoid expansion medium volume at 37°C every 3 days. Liver ductal organoid can be observed in 5-8 days.

4. Organoid passages

a. It is recommended to aspirate the upper medium and add 500 µL of Liver Ductal Organoid Basal Medium A. Use 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 liver ductal organoid is separated from MCE Basement Membrane Matrix using a pipette tip. Then collect the precipitate by centrifugation at 200-250 g for 3 min at room temperature.

c. Add 1 mL of Liver Ductal 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 an appropriate amount 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 3 min or less. The digestion is then terminated by adding 1 mL Liver Ductal Organoid Basal Medium A.

d. Centrifuge at 200-250 g for 3 min at room temperature. After centrifugation, the supernatant was discarded and washed 1-2 times with Liver Ductal Organoid Basal Medium A or PBS and then prepared for use.

e. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. Approximately 40-200 ducts should be plated in 50 µL Basement Membrane Matrix. The volume of Basement Membrane Matrix should be greater than 2/3 of the total diluted volume.

f. 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 gelling.

g. Inject 500 µL of liver ductal organoid maintenance 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 liver ductal organoid maintenance medium volume at 37°C every 3-4 days.

4 Storage

Individual Components	Mouse Liver Ductal Organoid (expansion) Basal Medium A	4°C, 1 year.
	Mouse Liver Ductal Organoid (expansion) Supplement B (50x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Mouse Liver Ductal Organoid (expansion) Supplement C (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
Complete Culture Medium	Mouse Liver Ductal Organoid (expansion) Complete Culture Medium	4°C, 2 weeks or -20°C, 3 months. 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. Primary tissue cells need to be kept sterile when extracted from primary 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.