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Mouse Colonic Organoid Kit

1 Contents

Cat.No.	Component	HY-K6120-100 mL	HY-K6120-500 mL
HY-K6120-A	Mouse Colonic Organoid Basal Medium A	100 mL	500 mL
HY-K6120-B	Mouse Colonic Organoid Supplement B (50x)	1 mL × 2	10 mL
HY-K6120-C	Mouse Colonic Organoid Supplement C (250x)	0.4 mL	1 mL × 2
HY-K6120-D	Mouse Colonic Organoid Supplement D (250x)	0.4 mL	1 mL × 2
HY-K6120-E	EDTA (0.5 M, PH 8.0)	0.2 mL	1 mL

2 Introduction

MCE Mouse Colonic Organoid Kit contains Colonic Organoid Basal Medium A, Colonic Organoid Supplement B (50x), Colonic Organoid Supplement C (250x), Colonic Organoid Supplement D (250x) and EDTA. This product can be used to efficiently construct mouse colonic organoid.

The stem cells and progenitor cells are located at the base of the intestinal mucosal crypts, and they have self-renew and differentiate into various mature cell types. Colonic organoids derived from primary tissue possess the ability to self-renew and mimic the characteristics of real colon tissue. Colonic organoids can be applied to study colon development, disease mechanisms, and drug screening.

3 Operation Instructions

1. Preparation of complete medium for colonic organoid

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

a. Prepare expansion medium for colonic organoid

Reagents	10 mL	20 mL	50 mL	Final concentration
Mouse Colonic Organoid Basal Medium A	9.72 mL	19.44 mL	48.6 mL	1x
Mouse Colonic Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Mouse Colonic Organoid Supplement C (250x)	40 µL	80 µL	200 µL	1x
Mouse Colonic Organoid Supplement D (250x)	40 µL	80 µL	200 µL	1x

b. Prepare differentiation medium for colonic organoid

Reagents	10 mL	20 mL	50 mL	Final concentration
Mouse Colonic Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Mouse Colonic Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Mouse Colonic 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 Mouse Colonic 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 2 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.

d. Add 10 mL of ice-cold 2 mM EDTA-PBS and place at 4°C on a horizontal shaker 30 min.

e. Stand the tube and let the samples settle by gravity. Remove the EDTA from the tube by aspiration, and gently add 10 mL of cold PBS to wash the samples.

f. In a culture dish or a new 50 mL tube containing cold PBS, pipette up and down using a 5 mL pipette to release the crypts. Take 10 μL and observe under a microscope to confirm the presence of a large number of intestinal crypts. Check the supernatant for crypt numbers in the first fraction.

g. Filter through a 70 μm filter into a new tube, then spin the crypts at 300 g for 3 min at 4°C, and aspirate the supernatant.

h. Resuspend the pellet in 1 mL PBS on ice and transfer into a new 1.5 mL tube.Count the number of crypts per 20 μL drop under a stereomicroscope. Centrifuge at 300 g for 3 min at 4°C to collect the pellet after counting.

3. Construction of organoid

a. Resuspend the collected crypts in MCE Basement Membrane Matrix on ice. Approximately 20-120 crypts should be plated in 10 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. 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 gelling.

c. Inject 500 μL of colonic 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 colonic organoid expansion medium volume at 37°C every 3 days. Colonic organoids can be observed in 5-7 days.

4. Organoid passages

a. It is recommended to aspirate the upper medium and add 500 μL of Mouse Colonic 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 colonic 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 4°C

c. Add an appropriate amount of organoid digestion solution in 37°C incubator to digest the organoid. The digestion time should be limited to 3 min or less. The digestion is then terminated by adding 1 mL Mouse Colonic Organoid Basal Medium A.

d. Centrifuge at 250-300 g for 3 min at 4°C. Aspirate the supernatant, and add Colonic Organoid Basal Medium A. Centrifuge at 250-300 g for 3 min at 4°C. Aspirate the supernatant and place tubes on ice.

e. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. 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. Use 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 gelling.

g. Inject 500 μL of colonic 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.

h. Closely monitor organoid formation. When the colonic organoid reaches a diameter larger than 100 μm, remove the colon organoid expansion medium and add 500 μL of colon organoid differentiation medium. Typically, it takes at least 4 days to differentiation. Change the medium every 2 days.

4 Storage

Individual Components	Mouse Colonic Organoid Basal Medium A	4°C, 1 year.
	Mouse Colonic Organoid Supplement B (50x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Mouse Colonic Organoid Supplement C (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Mouse Colonic Organoid Supplement D (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	EDTA (0.5M, PH 8.0)	Room temperature, 5 years
Complete Culture Medium	Mouse Colonic Organoid Expansion Complete Culture Medium	4°C, 2 weeks or -20°C, 3 months. Avoid repeated freeze/thaw cycles.
	Mouse Colonic Organoid Differentiation 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.

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.