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Mouse Intestinal Organoid Kit

1 Contents

Cat.No.	Component	HY-K6119-100 mL	HY-K6119-500 mL
HY-K6119-A	Mouse Intestinal Organoid Basal Medium A	100 mL	500 mL
HY-K6119-B	Mouse Intestinal Organoid Supplement B (50x)	1 mL × 2	10 mL
HY-K6119-C	Mouse Intestinal Organoid Supplement C (250x)	0.4 mL	1 mL × 2
HY-K6119-D	EDTA (0.5 M, pH 8.0)	0.2 mL	1 mL

2 Introduction

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

The stem cells (ISCs) 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. Intestinal organoids derived from primary tissue possess the ability to self-renew and mimic the characteristics of real colon tissue. Intestinal organoids can be applied to study colon development, disease mechanisms, and drug screening.

3 Operation Instructions

1. Preparation of complete medium for intestinal organoid

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

Reagents	10 mL	20 mL	50 mL	Final concentration
Mouse Intestinal Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Mouse Intestinal Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Mouse Intestinal 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 Intestinal 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. Transfer them to a 15 mL conical tube.

d. Add 10 mL of ice-cold 5 mM EDTA-PBS and place at 4°C on a horizontal shaker 30 minutes.

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 crypts.

g. Filter through a 70 µm filter into a new tube, then spin the crypts at 150-200 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 20-60 crypts should be plated in per 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 intestinal 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 intestinal organoid complete medium volume at 37°C every 3 days. Intestinal 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 Intestinal 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 intestinal organoid is separated from MCE Basement Membrane Matrix using a pipette tip. Then collect the precipitate by centrifugation at 150-200 g for 3 min at 4°C.

c. Add 1 mL of Mouse Intestinal Organoid Basal Medium A and resuspend and gently blow well until the organoids are dispersed into fragments.

d. Centrifuge at 150-200 g for 3 min at 4°C. Aspirate the supernatant, wash the pellet with Mouse Intestinal Organoid Basal Medium A or PBS.

e. Centrifuge at 150-200 g for 3 min at 4°C. Aspirate the supernatant and place tubes on ice.

f. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. Approximately 20-60 crypts should be plated in per 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.

g. 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.

h. Inject 500 µL of intestinal 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.

4 Storage

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