MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

MCE® MedChemExpress

Human Colonic Organoid Kit

1 Contents

Cat.No.	Component	HY-K6112-100 mL	HY-K6112-500 mL
HY-K6112-A	Human Colonic Organoid Basal Medium A	100 mL	500 mL
HY-K6112-B	Human Colonic Organoid Supplement B (50x)	1 mL × 2	10 mL
HY-K6112-C	Human Colonic Organoid Supplement C (250x)	0.4 mL	1 mL × 2
HY-K6112-D	Human Colonic Organoid Supplement D (250x)	0.4 mL	1 mL × 2

2 Introduction

MCE Human Colonic Organoid Kit contains Colonic Organoid Basal Medium A, Colonic Organoid Supplement B (50x), Colonic Organoid Supplement C (250x), and Colonic Organoid Supplement D (250x). This product can be used to efficiently construct human 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 organoid according to the following components, mix thoroughly and set aside on ice.

a. Prepare colonic organoid expansion medium for primary culture and resuscitation.

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

b. Prepare colonic organoid maintenance medium for the transfer of culture.

Reagents	10 mL	20 mL	50 mL	Final concentration
Human Colonic Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Human Colonic Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Human 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 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 containing 10 mL of pre-cooled PBS.

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. Aspirate the supernatant with a 10 mL pipette and add 10 mL of pre-cooled PBS. Repeat 3-5 times until the supernatant no longer contains any visible debris.

f. Aspirate the supernatant with a 10 mL pipette. Add an appropriate amount of pre-warmed Tissue Digestion Solution, 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 min.

g. Once crypt structures appeared, digestion should be stopped by adding FBS at a final concentration of 2%, then filtered using a 100 μm cell strainer and the filtrate should be collected.

h. Resuspend 10 mL of Human Colorectal Organoid Basal Medium A into a conical tube, then centrifuge at 250g for 3 minutes at 4°C to collect the cell precipitate. Repeat the procedure 1-2 times.

3. Construction of organoid

a. Resuspend the collected crypts in MCE Basement Membrane Matrix on ice. Approximately 50-200 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 solidification.

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 3-4 days. Colonic organoids can be observed in 5-8 days.

3. Organoid passages

a. It is recommended to aspirate the upper medium and add 500 μL of 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 200-250 g for 3 min at room temperature.

c. Add 1 mL of Colonic 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 Colonic Organoid Basal Medium A.

d. Centrifuge at 200-250 g for 3 min at room temperature. After centrifugation, the supernatant was discarded and washed with Colonic Organoid Basal Medium A and then prepared for use.

e. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. Approximately 50-200 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.

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 gelling.

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

4 Storage

Individual Components	Human Colonic Organoid Basal Medium A	4°C, 1 year.
	Human Colonic Organoid Supplement B (50x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Human Colonic Organoid Supplement C (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
Complete Culture Medium	Human Colonic Organoid Supplement D (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Human Colonic Organoid Expansion Complete Culture Medium	4°C, 2 weeks or -20°C, 3 months. Avoid repeated freeze/thaw cycles.
	Human Colonic Organoid Maintenance 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.