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Human Intestinal Organoid Kit

1 Contents

Cat.No.	Component	HY-K6113-100 mL	HY-K6113-500 mL
HY-K6113-A	Human Intestinal Organoid Basal Medium A	100 mL	500 mL
HY-K6113-B	Human Intestinal Organoid Supplement B (50x)	2 mL	10 mL
HY-K6113-C	Human Intestinal Organoid Supplement C (250x)	0.4 mL	2 mL
HY-K6113-D	Human Intestinal Organoid Supplement D (250x)	0.4 mL	2 mL

2 Introduction

MCE Human Intestinal Organoid Kit contains Human Intestinal Organoid Basal Medium A, Human Intestinal Organoid Supplement B (50x), Human Intestinal Organoid Supplement C (250x) and Human Intestinal Organoid Supplement D (250x). This product can be used to efficiently construct human intestinal organoids.

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. Intestinal organoids derived from primary tissue possess the ability to self-renew and mimic the characteristics of real intestine tissue. Intestinal organoids can be applied to study intestine development, disease mechanisms, and drug screening.

3 Operation Instructions

1. Preparation of complete medium for intestinal organoid

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

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

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

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

Reagents	10 mL	20 mL	50 mL	Final concentration
Human Intestinal Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Human Intestinal Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Human 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 basal medium 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 basal medium with 1% FBS.
d. Prepare basal medium 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 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. Stop digestion by adding FBS to a final concentration of 2% and pipetting gently up and down. Stand the tube and let the samples settle by gravity for 1-2 min. Collect the supernatant into a new tube.
h. Add 10 mL basal medium. 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.
i. Resuspend the collected cell precipitate by adding 10 mL basal medium, and then collect the cell precipitate by centrifugation at 300 g for 3 min at 4°C. Repeat this step twice.

3. Construction of organoid

a. Resuspend the collected crypts in MCE Basement Membrane Matrix on ice. Approximately 5-20 crypts should be plated in 1 μL MCE Basement Membrane Matrix. It is recommended that 100% MCE Basement Membrane Matrix be used to resuspend primary cells. If dilution is required, please ensure that the ratio of the volume of MCE Basement Membrane Matrix to the volume of organoid medium used for dilution is greater than 2:1.
b. 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.
c. Inject 500 μL of human intestinal 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 human intestinal organoid expansion medium volume at 37°C every 3-4 days. Intestinal organoids can be observed in 5-8 days.

4. Organoid passages

a. It is recommended to aspirate the upper medium and add 500 μL of basal medium. 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 200-250 g for 3 min at room temperature.
c. Add 1 mL of basal medium 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 until it is dispersed into cell clusters containing 10-50 cells. The digestion time should be limited to 3 min. The digestion is then terminated by adding 1 mL basal medium.
d. Centrifuge at 200-250 g for 3 min at room temperature. After centrifugation, the supernatant was discarded and washed 1-2 times with basal medium or PBS and then prepared for use.
e. Resuspend the collected primary cells in MCE Basement Membrane Matrix on ice. Approximately 5-20 crypts should be plated in 1 μL MCE Basement Membrane Matrix. It is recommended that 100% MCE Basement Membrane Matrix be used to resuspend primary cells. If dilution is required, please ensure that the ratio of the volume of MCE Basement Membrane Matrix 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 human intestinal 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 human intestinal organoid maintenance medium volume at 37°C every 3-4 days.

4 Storage

Individual Components	Human Intestinal Organoid Basal Medium A	4°C, 1 year.
	Human Intestinal Organoid Supplement B (50x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Human Intestinal Organoid Supplement C (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
	Human Intestinal Organoid Supplement D (250x)	-20°C, 1 year. Avoid repeated freeze/thaw cycles.
Complete Culture Medium	Human Intestinal Organoid Expansion Complete Culture Medium	4°C, 2 weeks or -20°C, 3 months. Avoid repeated freeze/thaw cycles.
	Human Intestinal 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. It is not recommended to leave the prepared complete medium in the refrigerator at 4°C for more than 2 weeks.
2. Primary cells need to be kept sterile when extracted from primary tumor tissue to avoid contamination from subsequent experiments.
3. Organoid Basal Medium A is preferably used exclusively for the preparation of complete organoid media and is not recommended for washing primary tissues and resuspending cells. Basal media for washing primary tissues and resuspending cells are all types of base media suitable for cell culture, preferably epithelial organoid basal media.
4. 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.
5. 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.
6. This product is for R&D use only, not for drug, household, or other uses.
7. For your safety and health, please wear a lab coat and disposable gloves to operate.