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Human Gastric Epithelial Organoid Kit

1 Contents

Cat.No.	Component	HY-K6114-100 mL	HY-K6114-500 mL
HY-K6114-A	Human Intestinal Organoid Basal Medium A	100 mL	500 mL
HY-K6114-B	Human Intestinal Organoid Supplement B (50x)	1 mL × 2	10 mL
HY-K6114-C	Human Intestinal Organoid Supplement C (250x)	0.4 mL	1 mL × 2
HY-K6114-D	Human Intestinal Organoid Supplement D (250x)	0.4 mL	1 mL × 2

2 Introduction

MCE Human Gastric Epithelial Organoid Kit contains Gastric Epithelial Organoid Basal Medium A, Gastric Epithelial Organoid Supplement B (50x), Gastric Epithelial Organoid Supplement C (250x), and Gastric Epithelial Organoid Supplement D (250x). This product can be used to efficiently construct human gastric epithelial organoid.

Human gastric epithelial organoids can provide useful models for studying stomach-related diseases and drug screening.

3 Operation Instructions

1. Preparation of complete medium for gastric epithelial organoid

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

a. Prepare gastric epithelial organoid expansion medium for primary culture and resuscitation.

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

b. Prepare gastric epithelial organoid maintenance medium for long-term culture.

Reagents	10 mL	20 mL	50 mL	Final concentration
Human Gastric Epithelial Organoid Basal Medium A	9.76 mL	19.52 mL	48.8 mL	1x
Human Gastric Epithelial Organoid Supplement B (50x)	200 µL	400 µL	1 mL	1x
Human Gastric Epithelial 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 Gastric Epithelial 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 1-3 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. Wash the samples by pipetting with a 10 mL pipette at least ten times. Stand the tube and let the samples settle by gravity.

e. 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 30-60 min. Every 5-8 min, pipette the mixture up and down.

f. Check digestion under the microscope if the epithelium cell clusters appear during digestion, and once it appears, stop digestion by adding FBS to a final concentration of 2% and pipetting gently up and down. Filter through a pre-wetted 100 μm cell strainer into a new tube on ice.

g. Collect the cell precipitate after centrifugation at 250 g for 5 min at 4°C using a cryogenic centrifuge. If the cell precipitate is red, aspirate the supernatant and resuspend with 2-4 mL of erythrocyte lysate, trying to avoid the pipette tip touching the bottom of the tube. Centrifuge at 4°C for 3 min at 250 g to collect the cell precipitate after lysis.

h. Resuspend the collected cell precipitate by adding an appropriate amount of Human Gastric Epithelial Organoid Basal Medium A, and then collect the cell precipitate by centrifugation at 250 g for 3 min at 4°C. Repeat this step 1-2 times.

3. Construction of organoid

a. Resuspend the pellet in MCE Basement Membrane Matrix on ice. Approximately 50-200 ducts should be plated in 50 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 gastric epithelial organoid primary culture 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 gastric epithelial organoid expansion medium at 37°C every 3-4 days. Gastric epithelial 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 Gastric Epithelial 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 Gastric Epithelial organoid 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 Human Gastric Epithelial 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 Human Gastric Epithelial Organoid Basal Medium A.

d. Centrifuge at 250-300 g for 3 min at room temperature. After centrifugation, the supernatant was discarded and washed with Human Gastric Epithelial Organoid Basal Medium A and then prepared for use.

e. Resuspend the pellet in MCE Basement Membrane Matrix on ice. Approximately 50-200 ducts should be plated in 50 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 gastric epithelial 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. Gastric epithelial organoid can be observed in 5-8 days.

4 Storage

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