bioGenous™ Mouse Colonic Organoid Kit (Serum-free)

Catalog: K2204-MC

Product Description

bioGenous™ Mouse Colonic Organoid Kit is a serum-free culture medium designed for the primary culture and differentiation of mouse colonic organoids. During the primary culture phase, the organoids are primarily composed of colonic stem cells (LGR5+) and progenitor cells. Upon differentiation, the mouse colonic organoids also contain colonic absorptive cells (VILLI+) and goblet cells (MUC2+). These organoids faithfully replicate the characteristics of the *in vivo* colonic epithelium in terms of self-renewal and differentiation capabilities, tissue architecture, cell type composition, and functionality. In summary, bioGenous™ Mouse Colonic Organoid Kit provides an ideal in vitro model for studying mouse colon biology.

Product Information

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Mouse Colonic Organoid Basal Medium	K2204-MC-A100/A500	100 mL/500 mL	4°C,12 months
bioGenous™ Mouse Colonic Organoid Supplement B (50x)	K2204-MC-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Mouse Colonic Organoid Supplement C (250x)	K2204-MC-C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Mouse Colonic Organoid Supplement D (250x)	K2204-MC-D100/D500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
EDTA (0.5 M, pH 8.0)	E219121	1 mL/2 mL	15 - 30°C,5 years

Materials & Reagents Required But Not Included

The following extended materials and reagents required for organoid maintenance can be purchased from www.biogenous.cn.

Manufacturer	Materials	Catalog#
bioGenous™	Anti-Adherence Rinsing Solution	E238002
bioGenous™	Organoid Cryopreservation Medium (Serum-free)	E238023
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
	DPBS (1x), liquid, contains no calcium or magnesium	-
	70 μm cell strainer	-
	Cell counting plate	
	24-well cell culture plate	
	Pipette and 0.2mL, 1mL, 5mL pipette tips	
	15mL, 50mL centrifuge tubes, 1.5mL EP tubes	

Safety Precautions

Always follow standard laboratory safety procedures when handling biological materials. Wear appropriate personal protective equipment (PPE), including gloves, lab coat, and eye protection. Dispose of waste materials according to local regulations.

Preparation Before Use

Before initiating the protocol, ensure that all components and equipment are properly prepared:

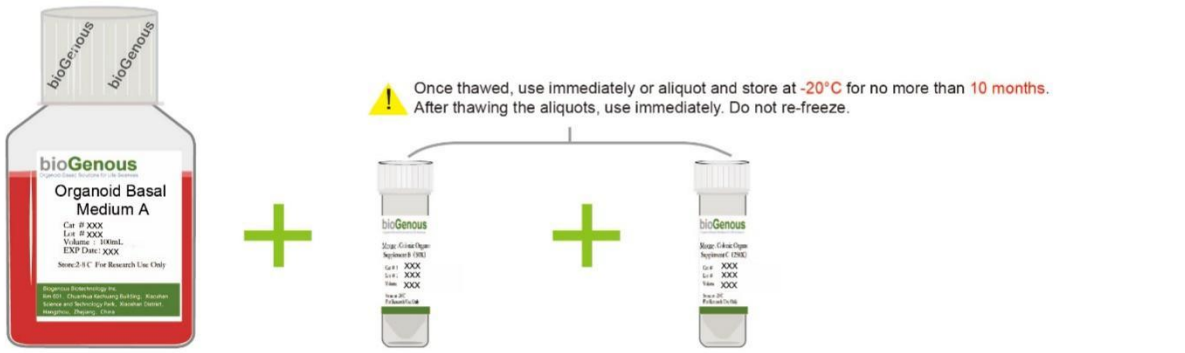
1. Verify that all components are stored according to the guidelines provided in the manual. Avoid repeated freeze-thaw cycles for sensitive reagents. Thaw all necessary reagents according to the instructions. Keep on ice or at the recommended temperature until ready to use.
2. Ensure that all equipment, such as incubators, pipettes, and centrifuges, are calibrated and functioning correctly.

Preparation of Preparation of Mouse Colonic Organoid Primary Culture Medium and Maintenance Medium

Using aseptic techniques, prepare the mouse colonic organoid primary culture medium and maintenance medium as follows. The example below outlines the preparation for 10 mL of primary culture medium and maintenance medium. Adjust volumes as necessary for different quantities.

Mouse Colonic Organoid Primary Culture Medium

Mouse Colonic Organoid Maintenance Medium



If not use immediately, store complete medium at 2-8°C for no more than 2 weeks.
bioGenous™ Mouse Colonic Organoid Supplement B contains fungicides and antibiotics (50x).

Mouse Colonic Organoid Primary Culture Medium and Maintenance Medium:

1. Thaw Mouse Colonic Organoid Supplement B (50x), Mouse Colonic Organoid Supplement C (250x) and Mouse Colonic Organoid Supplement D (250x) on ice. Mix thoroughly.

2. For **Mouse Colonic Organoid Primary Culture Medium** which used specifically for primary culture and resuscitation. Add 200 µL Mouse Colonic Organoid Supplement B (50x), 40 µL Mouse Colonic Organoid Supplement C (250x) and 40 µL Mouse Colonic Organoid Supplement D (250x) to 9.72 mL Mouse Colonic Organoid Basal Medium. Mix thoroughly.

3. For **Mouse Colonic Organoid Maintenance Medium** which used specifically for long-term and differentiation culture. Add 200 µL of Mouse Colonic Organoid Supplement B (50x) and 40 µL of Mouse Colonic Organoid Supplement C (250x) to 9.76 mL of Mouse Colonic Organoid Basal Medium. Mix thoroughly.

Protocol for Establishing Mouse Colonic Organoids

⚠ Studies involving primary mouse tissue material must follow all relevant institutional and governmental regulations. Informed consent must be obtained from all subjects before the collection of the primary mouse tissue material.

Establishment of Organoids from Primary Tissue

1. Collect primary mouse colonic biopsies in ice-cold Primary Tissue Storage Solution (K601005) using conical tubes. Keep tissue samples at 4°C until the start of the isolation.
2. Assess whether the obtained biopsies consist purely of epithelial tissues. If fat or muscle tissues are present, remove these non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. Otherwise, continue to the Step 3.
3. Rinse the tissue with Epithelial Organoid Basal Medium (B213152) or DPBS twice.
4. Mince the tissue into small fragments of 1-3 mm^3 in a cell culture dish using surgical scissors or scalpels.
5. Digest the tissue fragments with 10 mL of Tissue Digestion Solution (K601008) in a 15 mL conical tube at 37°C, with variable incubation times ranging from 30 min to 1 h. Carefully monitor the digestion process, mixing the content of the tube every 5-10 min by shaking vigorously or pipetting the mixture up and down. The digestion process is complete when most of tissue fragments could pass through the 1 mL pipette tips.
6. Terminate tissue digestion by adding FBS to the tissue digestion mixture to a final concentration of 2% and filter using a 100 μm cell strainer.
7. Collect and centrifuge the filtered cells at 250 x *g* for 3 min at 4°C. In case of a visible red pellet, aspirate the supernatant and resuspend the pellet using 2 mL of Red Blood Cell Lysis Solution (E238010) to lyse the erythrocytes at room temperature for 1 min and centrifuge at 250 x *g* for 3 min at 4°C.
8. Aspirate the supernatant and resuspend the pellet in Epithelial Organoid Basal Medium, centrifuge at 250 x *g* for 3 min at 4°C. Repeat this step once more time.
9. Aspirate the supernatant and resuspend the pellet in bioGenous™ Organoid Culture ECM (M315066). The ECM should be kept on ice to prevent solidification. The amount of ECM used depends on the size of the pellet. Approximately 10,000 cells should be plated in 25 μL of ECM.
   CRITICAL: *Do not overly dilute the ECM (>70% (ECM vol/total vol)), as this may inhibit the proper formation of the solid droplets.*
10. Seed the ECM containing cells at the bottom of 24-well cell culture plates in droplets of ~30 μL each around the center of the well.
    CRITICAL: *Once the cells are resuspended in ECM, proceed as quickly as possible, as the ECM may solidify in the tube or pipette tip. Do not let the ECM touch the walls of well.*
11. Prepare the required amount of mouse colonic organoid primary culture medium.
12. Once the ECM droplets have solidified (15-25 min) and carefully add 500 μL organoid primary culture medium to each well.
    CRITICAL: *Do not add the medium directly on top of the ECM droplets, as this might disrupt the droplets.*
13. Place the culture plate in a humidified incubator at 37°C and 5% (vol/vol) CO_2.
14. Change the medium every 3-4 days by carefully aspirating the medium from the wells and replacing it with a fresh, pre-warmed organoid primary culture medium.
15. Closely monitor the organoid formation. Ideally, mouse colonic organoids should be passaged for the first time between 7 and 10 days after the initial seeding. Typical morphologies of successfully cultured mouse colonic organoids are shown in Figure 1.

Splitting and Passaging of Organoids

1. Pipette up and down to disrupt the ECM and transfer the organoid suspension to a 1.5 mL tube. Continue pipetting up and down to create pressure to help remove the ECM.
2. Centrifuge the tube at 250 x *g* for 3 min at room temperature.
3. Aspirate the supernatant and split the organoids using either Organoid Dissociation Solution (E238001) or by mechanical disruption.

Organoid dissociation solution-based cell dissociation: Resuspend the pellet in 0.2 mL of Organoid Dissociation Solution, pipette up and down and incubate at 37°C until the organoids are released from the ECM. Pipette up and down with a filter tip for ≥8 times every 2 min to aid in the disruption of the organoids. Closely monitor the digestion to keep the incubation time in the Organoid Dissociation Solution to a minimum.

Mechanical disruption-based cell dissociation: Resuspend the pellet in 1.5 mL of Epithelial Organoid Basal Medium. Carefully pipette the organoid suspension up and down 30 times by pipetting against the bottom of the tube to create pressure, which will aid organoid disruption.

CRITICAL: *Do not dissociate in Organoid Dissociation Solution for >7 min, as this may result in poor organoid outgrowth or even loss of the culture. As a rule of thumb, digestion is complete if a mixture of small lumps of cells (consisting of 10-50 cells) can be observed.*

4. After shearing is complete, wash once by adding 1 mL Epithelial Organoid Basal Medium and centrifuge at 250 x *g* for 3 min at room temperature.
5. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) ECM, and plate organoids in droplets at the bottom of a culture plate. After seeding, transfer the culture plates to a humidified incubator at 37°C and 5% (vol/vol) CO_2 for 15-25 min.
6. Pre-warm the mouse colonic organoid maintenance medium at 37°C.
7. After the ECM droplets have solidified (15-25 min), carefully pipette the pre-warmed medium into the wells.
8. Place the culture plates in a humidified incubator at 37°C and 5% (vol/vol) CO_2 until the organoids are needed for further experiments.

Applications

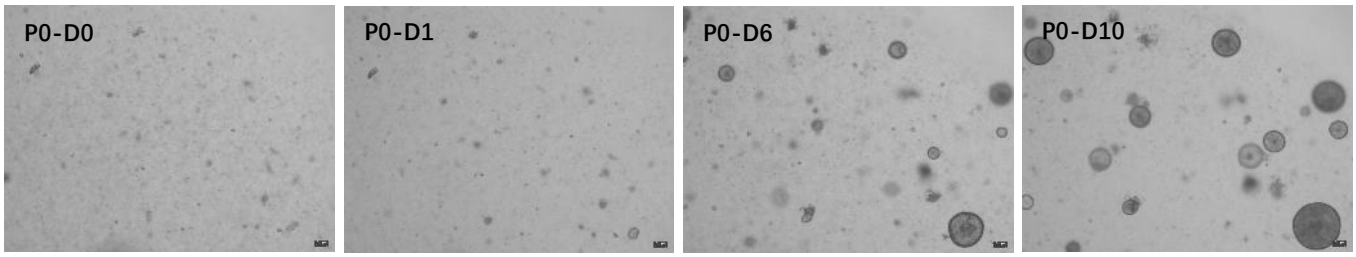


Figure 1. Morphological examples of primary culture of mouse colonic organoid in primary culture medium. Passage number, as well as days post embedding in the passage, are indicated below each image. Scale bar: 100 μm.

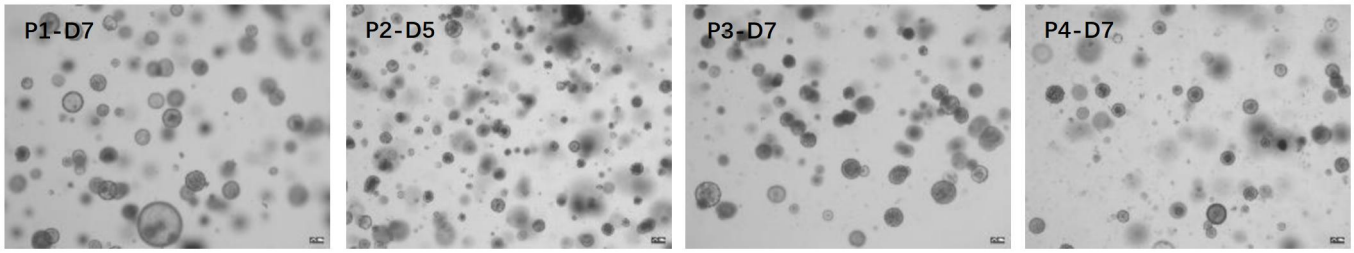


Figure 2. Representative images of mouse colonic organoid across different passages in maintenance medium. Passage number, as well as days post embedding in the passage, are indicated below each image. Scale bar: 100 μm.

Quality Control

All components are negative for bacterial and fungal contamination. Certificate of authenticity (COAs) for all other products are available upon request.

Safety information

Read the Safety Data Sheets (SDSs) and follow the manufacture's instruction.

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Contact and Support

For questions, suggestions, and technical supports, please contact us at E-mail: info@biogenous.cn.

Last updated on 30th August, 2024