bioGenous™ Mouse Intestinal Organoid Kit (Serum-free)

Catalog： K2001-MI

Product Description

bioGenous™ Mouse Intestinal Organoid Kit is a serum-free culture medium designed for mouse intestinal organoids derived from adult intestinal stem cells. Organoids grown in this complete medium predominantly consist of intestine stem cells (LGR5+), rapidly proliferating cells (KI67+), absorptive enterocytes (ALPi+), Paneth cells (ATOH1+), and goblet cells (MUC2+). These mouse intestinal organoids faithfully recapitulate the characteristics of in vivo intestinal epithelium in terms of self-renewal capacity, tissue architecture, cell type composition, and functionality. Therefore, they serve as an ideal in vitro model for studying intestinal homeostasis and disease mechanisms.

Product Information

Component	Cat#	Volume	Storage & Stability
bioGenous™ Mouse Intestinal Organoid Basal Medium	K2001-MI-A100/A500	100 mL/500 mL	2-8°C, 12 months
bioGenous™ Mouse Intestinal Organoid Supplement B (50x)	K2001-MI-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles,12 months
bioGenous™ Mouse Intestinal Organoid Supplement C (250x)	K2001-MI-C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
EDTA (0.5M, pH 8.0)	E219121	0.2 mL/1 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	F238002
bioGenous™	Organoid Cryopreservation Medium（Serum Free）	F238023
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
	DPBS (1x), liquid, contains no calcium or magnesium	-
	70 um cell strainer	-

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.

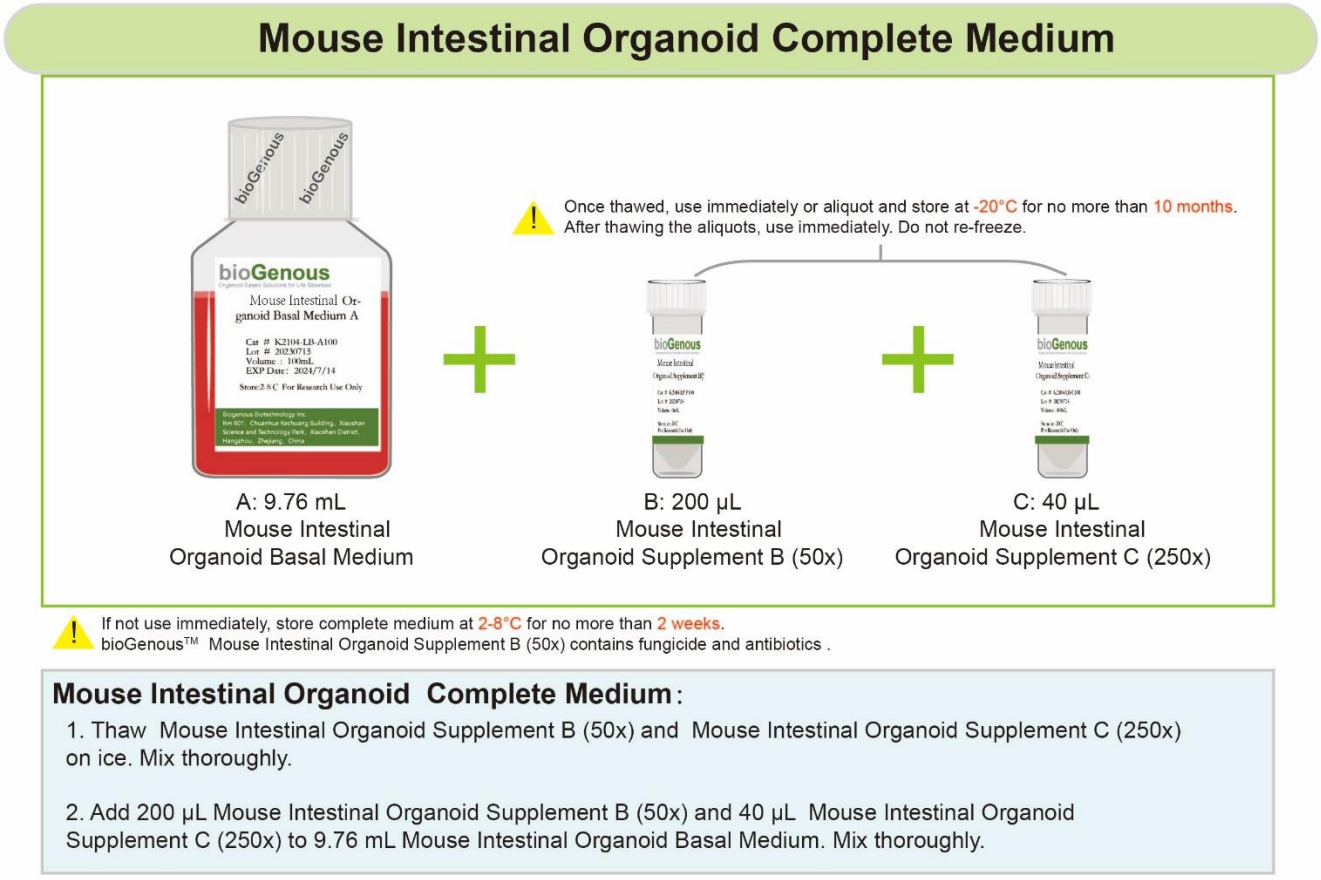
Preparation Before Use

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 Mouse Intestinal Organoid Complete Medium

Using aseptic techniques, prepare the mouse intestinal organoid complete medium as follows. The example below outlines the preparation for 10 mL of complete medium. Adjust volumes as necessary for different quantities.



Protocol for Establishing Mouse Intestinal 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. Prepare several culture dishes and add pre-cooled DPBS at 4°C for later use.
2. Perform standard surgical procedures to obtain segments of mouse small intestine, approximately 3 cm to 20 cm in length, and place them in culture dishes containing DPBS.
3. Use a pipette or syringe to inject DPBS into one end of the intestine to flush out the intestinal contents. After flushing, transfer the segment to a new culture dish with DPBS, and repeat the flushing process several times until the contents are completely removed. Transfer the cleaned intestine to a new dish containing DPBS.
4. Using surgical scissors, open the intestinal tube with the lumen facing up. Hold one end of the intestinal tissue with surgical forceps, and use a surgical blade to gently scrape off the intestinal mucosa. After the mucosa has been removed, transfer the tissue to a new dish with DPBS for washing, and repeat the washing process once.
5. Cut the washed intestinal tissue into pieces approximately 2 mm wide and transfer them to pre-cooled DPBS containing 5 mmol/L EDTA for digestion. Incubate at 4°C for 30 min.
6. After digestion, transfer the tissue fragments to a new dish containing DPBS for washing, and repeat once to remove EDTA.
7. Using a 5 mL pipette, gently pipette and resuspend the tissue fragments in a dish or 15 mL centrifuge tube containing

cold DPBS. This process generates mechanical shear forces that aid in the separation of crypts from the basal layer. Examine a portion of the suspension under a microscope. Once a large number of crypt-like structures are observed, stop pipetting and filter the suspension through a 70 μm mesh filter.

8. Collect the filtered tissue suspension and centrifuge at 150 x *g*, 4°C for 3 min.
9. Discard the supernatant and resuspend the tissue pellet in 1 mL DPBS. Take 20 μL of the suspension for microscopy and crypt counting. After counting, aspirate the volume containing the required number of crypts, centrifuge again at 150 x *g*, 4°C for 3 min, and place the pellet on ice.
10. Resuspend the tissue pellet in an appropriate volume of bioGenous™ Organoid Culture ECM (M315066). The recommended resuspension density is 200 to 600 crypts per 10 μL ECM suspension. Keep the resuspended ECM on ice, and complete the resuspension within 30 seconds to prevent premature ECM solidification.

    Note: The ECM dilution ratio should be 70% or higher to ensure structural stability during culture.
11. Deposit 30 μL of the ECM - cell suspension mixture into the center of each well of a 24-well plate, avoiding to touch the walls of well.

    Note: To prevent ECM from solidifying at room temperature, this step should be completed as quickly as possible**.**
12. Place the coated 24-well plate in a 37°C CO_2 incubator and incubate for approximately 20 minutes until the ECM solidifies.
13. Prepare the mouse intestinal organoid complete medium.
14. Once the ECM has fully solidified, add the prepared mouse intestinal organoid complete medium to each well of the 24-well plate, 500 μL per well.

    Note: Add the medium slowly along the walls to avoid disrupting the solidified ECM.
15. Place the 24-well plate in a 37°C CO_2 incubator for culture.
16. Change the medium every 3 days, taking care to avoid disrupting the ECM during the change.
17. Monitor the organoid growth closely. Ideally, the mouse intestinal organoids should develop within 5 to 7 days.

Passaging of Organoids

1. Using a pipette tip rinsed with the Anti-Adherence Rinsing Solution (E238002), gently pipette and transfer the organoids and culture medium suspension into a 1.5 mL EP tube that has also been rinsed with the Anti-Adherence Rinsing Kit.
2. With a pipette tip rinsed using the Anti-Adherence Rinsing Solution, vigorously resuspend the organoid suspension to separate the organoids from the ECM.
3. Centrifuge the suspension at 150 x *g*, 4°C for 3 min. Discard the supernatant, resuspend the organoid pellet in DPBS, centrifuge again at 150 g x *g*, 4°C for 3 min, discard the supernatant, and place the pellet on ice.
4. Resuspend the organoid pellet in an appropriate volume of ECM. Keep the resuspended ECM on ice, and complete the resuspension within 30 seconds to prevent premature ECM solidification.

    Note: The ECM dilution ratio should be 70% or higher to ensure structural stability during culture.
5. Deposit 30 μL of the ECM and organoid mixture into the center of each well of a 24-well plate, avoiding contact with the sides of the wells.

    Note: Complete this step promptly to prevent ECM from solidifying at room temperature.
6. Place the 24-well plate in a 37°C CO_2 incubator and incubate for approximately 15 min until the ECM solidifies.
7. Prepare the mouse intestinal organoid complete medium.
8. Once the ECM has fully solidified, add the prepared mouse intestinal organoid complete medium to each well of the 24-well plate, 500 μL per well.
9. Place the 24-well plate in a 37°C CO_2 incubator for culture.

Applications

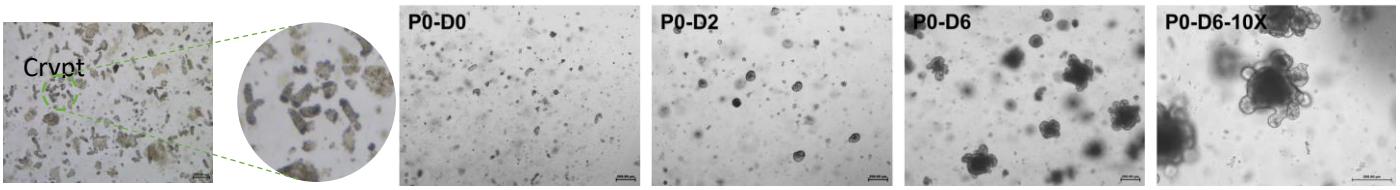


Figure 1. Examples of successful culture in primary culture of mouse intestinal organoid. Scale bar: 200 μm.

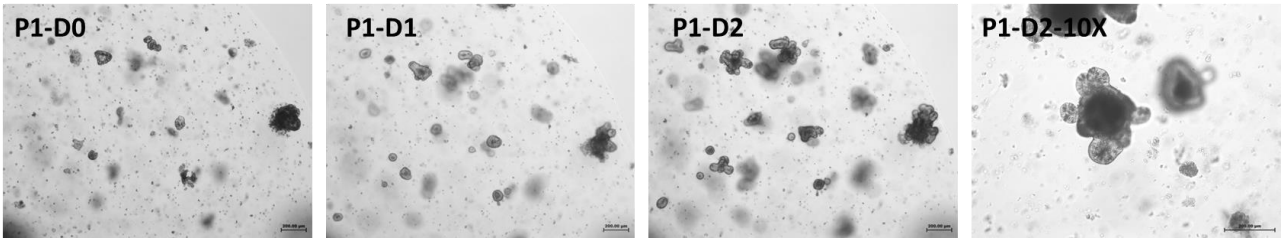


Figure 2. Examples of successful culture of passaged mouse intestinal organoids. Scale bar: 200 μm.

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.

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