bioGenous™ Mouse Intestinal Organoid Kit Plus (Serum-free)

Catalog: K2001P-MI

Product Description

bioGenous™ Mouse Intestinal Organoid Kit Plus is a comprehensive system designed for the generation and maintenance of mouse intestinal organoids derived from adult stem cells. This kit provides a complete set of reagents for isolating crypts from the intestine, recovering crypts, analyzing crypt activity, and culturing organoids. This kit effectively preserves crypt activity and enhances the formation rate of intestine organoids. The mouse intestinal organoids consist of intestine stem cells (LGR5+), rapidly proliferating cells (Ki67+), absorptive enterocytes (ALPi+), Paneth cells (Atoh1+), and goblet cells (MUC2+). These organoids faithfully recapitulate the features of the *in vivo* intestinal epithelium in terms of self-renewal capacity, tissue structure, cell types, and function, making them an ideal in vitro model for studying intestinal homeostasis and disease mechanisms.

Product Information

Component	Cat#	Volume	Storage & Stability
Intestine Crypt Dissociation Solution	K2001P-MI-I	20 mL/100 mL	2-8°C, 18 months
Intestine Crypt Recovery Solution	K2001P-MI-II	100 mL/500 mL	2-8°C, 18 months
bioGenous™ Mouse Intestinal Organoid Basal Medium	K2001P-MI-A100/ A500	100 mL/500 mL	2-8°C, 18 months
bioGenous™ Mouse Intestinal Organoid Supplement B (50x)	K2001P-MI-B100/ B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 18 months
bioGenous™ Mouse Intestinal Organoid Supplement C (250x)	K2001P-MI-C100/ C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 18 months
Trypan Blue Staining Solution (2x)	K601009	0.2 mL/1 mL	4-30°C, 24 months

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	

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.

Mouse Intestinal Organoid Complete Medium

A: 9.76 mL
Mouse Intestinal
Organoid Basal Medium A

B: 200 µL
Mouse Intestinal
Organoid Supplement B (50x)

C: 40 µL
Mouse Intestinal
Organoid Supplement C (250x)

Once thawed, use immediately or aliquot and store at -20°C for no more than 10 months. After thawing the aliquots, use immediately. Do not re-freeze.

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

Mouse Intestinal Organoid Complete Medium:

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

2. Add 200 µL Mouse Intestinal Organoid Supplement B (50x) and 40 µL Mouse Intestinal Organoid Supplement C (250x) to 9.76 mL Mouse Intestinal Organoid Basal Medium. Mix thoroughly.

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 Mouse Intestinal Organoid from Primary Tissue

1. Prepare several cell culture dishes and add an appropriate volume of pre-cooled DPBS (4°C) for use. Thaw Organoid Culture ECM (M315066, hereinafter referred to as ECM) on ice.
2. Perform standard surgical procedures to obtain mouse intestinal segments. For experimental purposes, select segments approximately 3-20 cm in total length and place them in culture dishes containing DPBS.
3. Use a pipette or syringe to inject DPBS into one end of the intestinal segment to flush out intestinal contents. After flushing, place the segment in a new culture dish containing DPBS and repeat the flushing process several times until the contents are completely washed out. Transfer the cleaned intestinal tissue to a new culture dish with DPBS.
4. Using surgical scissors, open the intestinal tube with the luminal surface facing up. Using one hand with surgical forceps to hold one end of the tissue, use a surgical blade to gently scrape the intestinal villi from the luminal surface. Once the villi are removed, transfer the tissue to a new culture dish with DPBS, and repeat the washing step.
5. Cut the washed intestinal tissue into 2 mm pieces and transfer them to a culture dish containing 4 mL of mouse crypt isolation solution. Incubate at 4°C for 30 min.
6. After incubation, transfer the intestinal tissue fragments to a new dish containing 3-4 mL of crypt recovery solution. Gently wash the tissue and repeat this step once more. Transfer the washed tissue fragments to a 15 mL or 50 mL centrifuge tube containing 5 mL of crypt recovery solution.

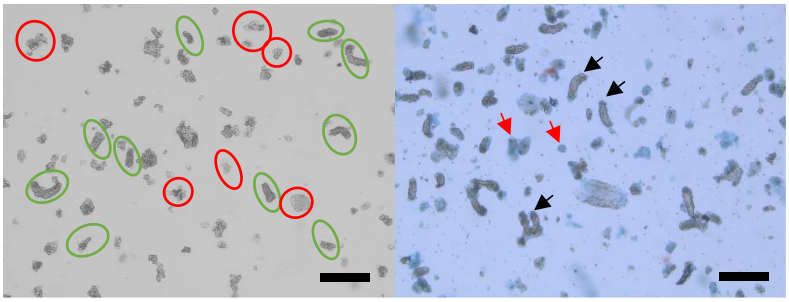
7. Use a 5 mL pipette to gently triturate the tissue fragments, allowing the tissue to pass through the pipette tip to generate mechanical shear force and separate the crypts from the intestinal base layer. After 5-10 triturations, take a small aliquot of the suspension for microscopic examination. Stop trituration when a large number of crypt-like structures are visible, and filter the triturated suspension through a 70 μm mesh filter.
8. Collect the filtrate and centrifuge at 150 x *g* for 3 min at 4°C.
9. Discard the supernatant and resuspend the tissue pellet in 1-3 mL of crypt recovery solution. Mix thoroughly. Take 10 μL of the suspension, mixing with an equal volume of Trypan Blue Staining Solution. Load onto a hemocytometer and observe under a microscope or cell counter to determine the proportion of viable cells and the number of intact crypts. Blue cells indicate dead cells, while transparent, unstained cells indicate live cells (Figure 1B).
10. Calculate the cell viability (cell viability = number of live cells / total number of cells × 100%). A viability greater than 80% indicates good separation, while a viability below 30% suggests significant tissue damage during separation, potentially leading to a lower primary organoid formation rate. Adjust the trituration force and number of triturations accordingly. Intact crypts typically appear as elongated structures (approximately 75-100 μm long and 25-40 μm wide); an example of primary isolated crypts is shown in Figure 1A).
11. After viability analysis, centrifuge the required volume of crypts or cell suspension at 150 x *g* for 3 min at 4°C. After discarding the supernatant, place the pellet on ice (this step should not exceed 5 min).
12. Resuspend the tissue pellet in an appropriate volume of ECM. A recommended resuspension density is 200 to 600 crypts per 10 μL of ECM solution. Keep the suspension on ice and avoid resuspending for more than 30 seconds to prevent premature ECM solidification.
    ***Note**: The ECM dilution ratio should be at 70% or higher to ensure structural stability during culture.*
13. Dispense the ECM - cell suspension into the center of each well of a 24-well cell culture plate, approximately 30 μL per well, avoiding contact with the sides of the wells.
    ***Note**: To prevent ECM from solidifying at room temperature, this step should be completed as quickly as possible.*
14. Place the culture plate in a 37°C CO_2 incubator and incubate for approximately 15 min, or until the ECM has solidified.
15. Once the ECM is completely solidified, add the prepared mouse intestinal organoid complete medium, 500 μL per well in the 24-well plate.
    ***Note**: Add the medium slowly along the walls to avoid disrupting the solidified ECM.*
16. Place the 24-well culture plate in a 37°C CO_2 incubator for culture.
17. Change the medium every 3 days, taking care to avoid disrupting the ECM during the process. Closely monitor organoid growth. Ideally, mouse intestinal organoids should develop within 5 to 7 days. An example of primary cultured Mouse Intestinal Organoids is shown in Figure 2.

Passaging and Differentiation of Mouse Intestinal Organoid

1. Use pipette tips rinsed with Anti-Adherence Rinsing Solution (E238002) to gently collect the organoids from the ECM. Transfer the organoids and medium suspension into a 1.5 mL EP tube, also rinsed with the Anti-Adherence Rinsing Solution.
2. Centrifuge at 150 x *g* for 3 min at 4°C. Discard the supernatant, resuspend the organoid pellet in DPBS, and centrifuge again at 150 x *g* for 3 min at 4°C. After discarding the supernatant, keep the pellet on ice.
3. Resuspend the organoid pellet in an appropriate volume of ECM. Keep the suspension on ice and ensure the resuspension time does not exceed 30 seconds to prevent premature ECM solidification.
    ***Note**: The ECM dilution ratio should be at 70% or higher to ensure structural stability during culture.*
4. Dispense the ECM-organoid suspension into the center of each well in a 24-well cell culture plate, approximately 30 μL per well, avoiding the ECM touching the walls of well.
    ***Note**: To prevent ECM from solidifying at room temperature, complete this step as quickly as possible.*
5. Place the culture plate in a 37°C CO_2 incubator and incubate for approximately 15 min or until the ECM has solidified.
6. Once the ECM is fully solidified, add the prepared Mouse Intestinal Organoid Complete Medium, 500 μL per well in the 24-well plate.
7. Place the 24-well culture plate in a 37°C CO_2 incubator for culture. An example of passaged mouse intestinal organoids is shown in Figure 3.

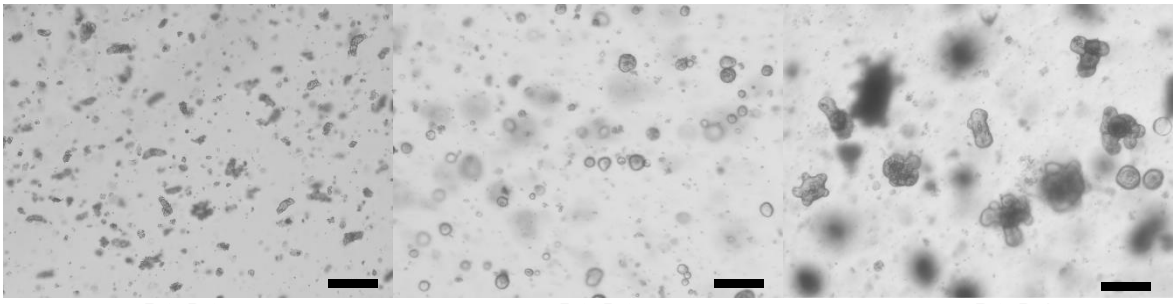
Applications

Examples of primary Mouse Intestinal crypts isolated using Mouse Intestinal Organoid Kit Plus.

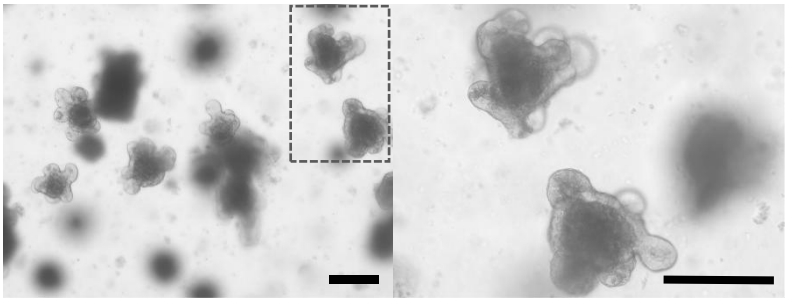


A Crypt B Crypt -Trypan Blue

Figure 1: Examples of primary mouse intestinal crypts isolated using the Mouse Intestinal Organoid Kit Plus. (A) Crypts with standard morphology outlined in green circles, while other clusters of heterogeneous cells are circled in red. (B) Viable crypts indicated by black arrows, and dead or poorly maintained crypts and cell clusters indicated by red arrows. Scale bar: 200 μm.

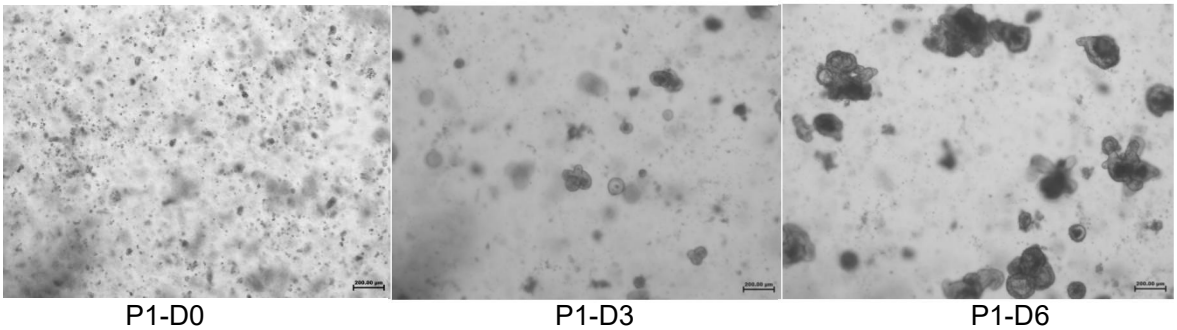


P0-D0 P0-D2 P0-D4



P0-D6 P0-D6 Enlarged

Figure 2: Examples of primary mouse intestinal organoids cultured using the Mouse Intestinal Organoid Kit Plus on days 0, 2, 4, and 6, and an enlarged view on day 6. Scale bar: 200 μm.



P1-D0 P1-D3 P1-D6

Figure 3: Examples of passaged mouse intestinal organoids cultured using the Mouse Intestinal Organoid Kit

Plus on days 0, 3, and 6. 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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