bioGenous™ Mouse Intestinal Organoid Kit with Wnt Optimization

Catalog: K2501-MIW

Product Description

bioGenous™ Mouse Intestinal Organoid Kit with Wnt Optimization is a flexible and efficient serum-free culture system designed for the robust establishment and long-term maintenance of mouse intestinal organoids. Compared with the previously released Mouse Intestinal Organoid Kit (Cat# K2001-MI), this upgraded version independently provides an optional Wnt ligand supplement (Component D), enabling researchers to adjust Wnt signaling intensity according to specific experimental requirements. The Wnt Supplement (Component D) can be selectively included during critical proliferative stages—such as early stem cell expansion or organoid establishment from single cells—to enhance crypt budding, stemness maintenance, and survival efficiency. When Paneth cells are absent or endogenous Wnt secretion is limited, supplementation with Component D effectively supports early organoid formation. Once stable organoid structures have developed, removing Component D promotes differentiation into mature intestinal organoids composed of LGR5+ intestinal stem cells, KI67+ proliferative cells, ALPi+ absorptive enterocytes, ATOH1+ Paneth cells, and MUC2+ goblet cells.

Product Information

Component	Cat#	Volume	Storage & Stability
Mouse Intestinal Organoid Basal Medium A	K2501-MIW-A100/A500	100 mL/500 mL	2-8°C, 12 months
Mouse Intestinal Organoid Supplement B (50X)	K2501-MIW-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles,12 months
Mouse Intestinal Organoid Supplement C (250X)	K2501-MIW-C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
Mouse Intestinal Organoid Supplement D (Wnt/β-catanin ligand, 2.5μg/mL)	K2501-MIW-D100/D500	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™	Organoid Culture ECM (Reduced Growth Factor)	M315066
bioGenous™	Epithelial Organoid Basal Medium (Serum-free)	B213151
bioGenous™	Organoid Cryopreservation Medium (Serum-free)	E238023
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Anti-Adherence Rinsing Solution	E238002
	DPBS (1X), liquid, contains no calcium or magnesium	-
	70 μm 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.
For research use only, not for use in diagnostic procedures.

Preparation Before Use

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

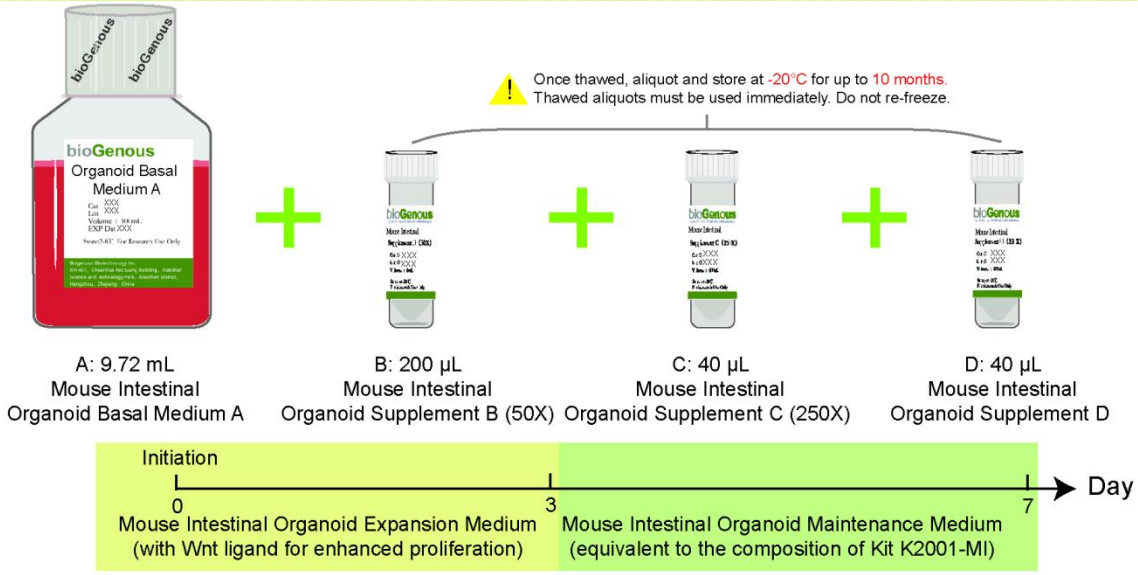
1. Verify that all components are stored according to the guidelines provided in the manual. Avoid repeated freeze-thaw cycles for sensitive reagents. Thaw all necessary reagents according to the instructions. Keep on ice or at the recommended temperature until ready to use.
2. Ensure that all equipment,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

Preparation of Mouse Intestinal Organoid Complete Medium

Use sterile technique to prepare the mouse intestinal organoid complete medium. Two formulations are available: Maintenance Medium (A+B+C) for long-term culture, and Expansion Medium (A+B+C+D) for rapid proliferation. The following examples describe the preparation of 10 mL of each medium; adjust the component volumes proportionally if preparing different amounts.

1. Thaw Mouse Intestinal Organoid Supplement B (50X) and Mouse Intestinal Organoid Supplement C (250X) on ice. For ABC + D medium, also thaw Mouse Intestinal Organoid Supplement D (250X) on ice.
   NOTE: *Once thawed, aliquot and store at -20°C for up to 10 months. Thawed aliquots must be used immediately. Do not re-freeze. If not used immediately, store the complete medium at 2–8°C for up to 2 weeks. bioGenous™ Mouse Intestinal Organoid Supplement B contains fungicide and antibiotics (50X).*
2. For Maintenance Medium (equivalent to the composition of Kit K2001-MI): Add 200 µL of Supplement B (50X) and 40 µL of Supplement C (250X) to 9.76 mL of Basal Medium A. Mix thoroughly. This formulation supports routine maintenance and long-term culture of mouse intestinal organoids.
   TIP: *This formulation is suitable for routine maintenance and long-term culture of mouse intestinal organoids.*
3. For Expansion Medium (with Wnt ligand for enhanced proliferation): Add 200 µL of Supplement B (50X), 40 µL of Supplement C (250X), and 40 µL of Supplement D (250X) to 9.72 mL of Basal Medium A. Mix thoroughly. This formulation is recommended for the first 2–3 days of primary crypt seeding or single-cell passaging to accelerate stem cell expansion and crypt budding (final Wnt concentration ~10 ng/mL; usable range 0.5–10 ng/mL). After initial expansion, switch to the Maintenance Medium to promote differentiation and maturation.
   NOTE: *This formulation is recommended for the first 3 days of primary crypt seeding or single-cell passaging to accelerate stem cell expansion and crypt budding (delivers ~10 ng/mL final Wnt ligand concentration; recommended range: 0.5-10 ng/mL; adjust D volume for 250X-1000X dilution as needed). After 3 days, switch to standard ABC medium to support differentiation and long-term maturation.*

Mouse Intestinal Organoid Expansion Medium (with Wnt ligand for enhanced proliferation)



If not used immediately, store the complete medium at 2–8°C for up to 2 weeks.
bioGenous™ Mouse Intestinal Organoid Supplement B (50X) contains fungicide and antibiotics .

Mouse Intestinal Organoid Expansion Medium (with Wnt ligand for enhanced proliferation)

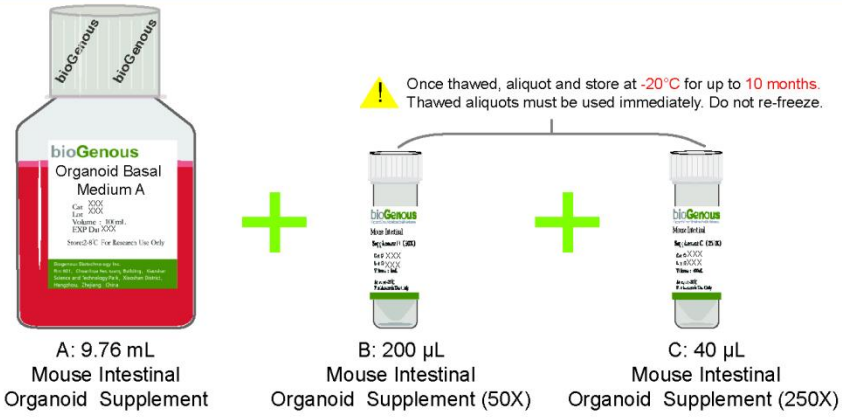
1. Thaw Mouse Intestinal Organoid Supplement B (50X), Mouse Intestinal Organoid Supplement C (250X) and Mouse Intestinal Organoid Supplement D (Wnt/β-catanin ligand, 2.5 µg/mL) on ice. Mix thoroughly.

2. .For Expansion Medium (with Wnt ligand for enhanced proliferation): This formulation is recommended for the first 2–3 days of primary crypt seeding or single-cell passaging to accelerate stem cell expansion and crypt budding.

Example: Add 200 µL Mouse Intestinal Organoid Supplement B (50X), 40 µL Mouse Intestinal Organoid Supplement C (250X) and 40 µL Mouse Intestinal Organoid Supplement D (Wnt/β-catanin ligand, 2.5 µg/mL) to 9.72 mL Mouse Intestinal Organoid Basal Medium. Mix thoroughly.
***NOTE**: Delivers ~10 ng/mL final Wnt ligand concentration; recommended range: 0.5-10 ng/mL; Adjust D volume for 250X-1000X dilution as needed.*

3. After 3 days, switch to standard ABC medium to support differentiation and long-term maturation.

Mouse Intestinal Organoid Maintenance Medium



If not used immediately, store the complete medium at 2–8°C for up to 2 weeks.
bioGenous™ Mouse Intestinal Organoid Supplement B (50X) contains fungicide and antibiotics .

Mouse Intestinal Organoid Maintenance Medium :

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

2. For Mouse Intestinal Organoid Maintenance Medium (equivalent to the composition of Kit K2001-MI): This formulation is suitable for routine maintenance and long-term culture of mouse intestinal organoids.
Add 200 µL of Supplement B (50X) and 40 µL of Supplement C (250X) to 9.76 mL of Basal Medium A. 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 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.
    CRITICAL: *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.
    CRITICAL: *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. Determine whether to include Supplement D based on crypt integrity: if the isolated crypts are abundant and morphologically intact, use the Maintenance Medium (A+B+C); if the crypt yield is low, fragmented, or derived from single cells, use the Expansion Medium (A+B+C+D) for the first 2–3 days to promote proliferation and survival.
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.
    CRITICAL: *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 (switch to medium without D after initial 2-3 days to promote differentiation), 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 bioGenous™ 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 x *g*, 4°C for 3 min, discard the supernatant, and place the pellet on ice.
   NOTE: *For single-cell organoid passaging, digest organoids into single cells using Organoid Dissociation Solution, then culture in Expansion Medium (A+B+C+D) for re-establishment. Once new organoid structures form, switch to Maintenance Medium.*
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.
   CRITICAL: *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.
   CRITICAL: *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 37°C CO_2 incubator for further culture and subsequent experimental use.

Applications

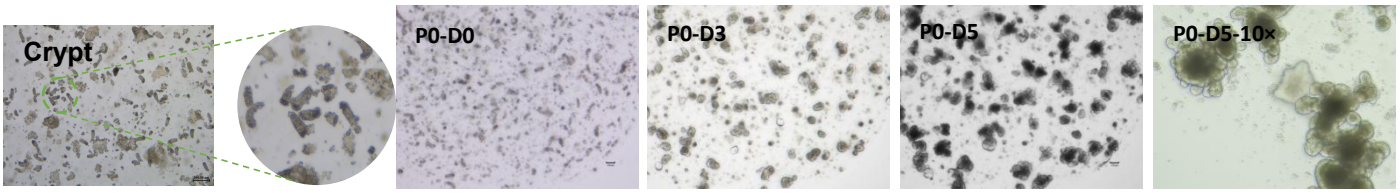


Figure 1. Examples of successful primary culture of mouse small intestinal organoids using Expansion Medium. Scale bar: 200 μm.

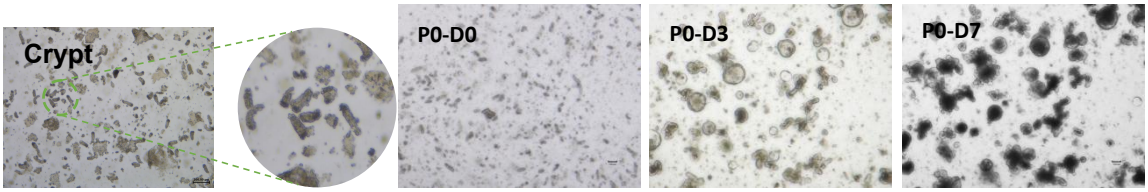


Figure 2 . Examples of successful primary culture of mouse small intestinal organoids using Maintenance Medium.Scale bar: 200 μm.

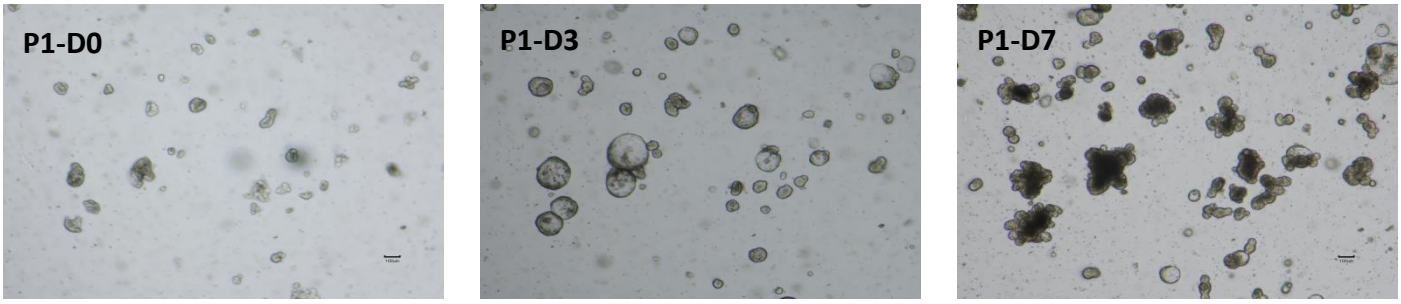


Figure 3 . Examples of successful continuous culture of passaged mouse small intestinal organoids using Maintenance Medium Scale bar: 100 μm.

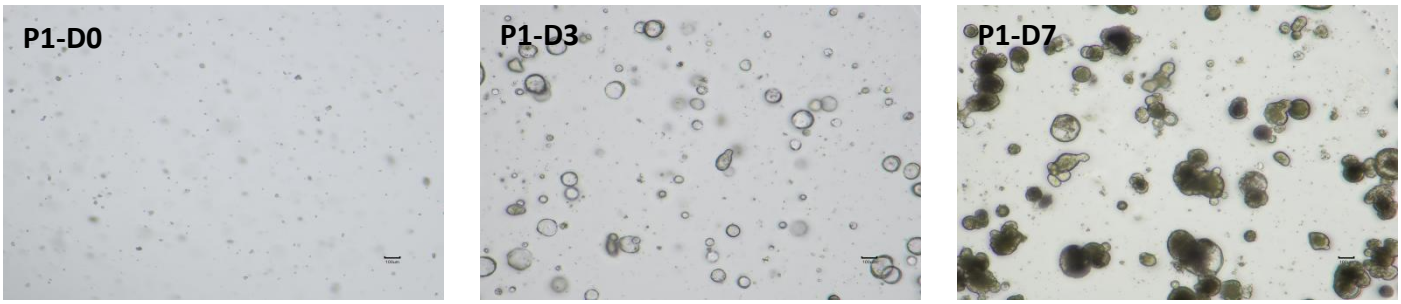


Figure 4 . Examples of successful single-cell culture of mouse small intestinal organoids using Expansion Medium. Scale bar: 100 μm.

Quality Control

All components are negative for bacterial and fungal contamination. Certificates 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 7-Nov-2025