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

Catalog: K2502-MIP

Product Description

bioGenous™ Mouse Intestinal Organoid Prime Kit is a defined, serum-free, 2-component culture system. Compared with the previous 3-component formulation, it greatly simplifies preparation procedures, improves stability and traceability, and enables efficient establishment and long-term maintenance of mouse small intestinal organoids. Through systematic optimization of the ratios of key factors and supportive environment in the culture system, the Prime version significantly enhances operational convenience, crypt budding efficiency, organoid formation speed, passaging expansion stability, and inter-batch reproducibility. It is suitable for intestinal stem cell research, epithelial regeneration studies, tumor modeling, gene editing, drug discovery and other related applications.

Product Information

Component	Cat#	Volume	Storage & Stability
Mouse Intestinal Organoid Basal Medium A	K2502-MIP-A100/A500	100 mL/500 mL	2-8 °C, 12 months
Mouse Intestinal Organoid Supplement B (50×)	K2502-MIP-B100/B500	2 mL/10 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 (1×), 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.

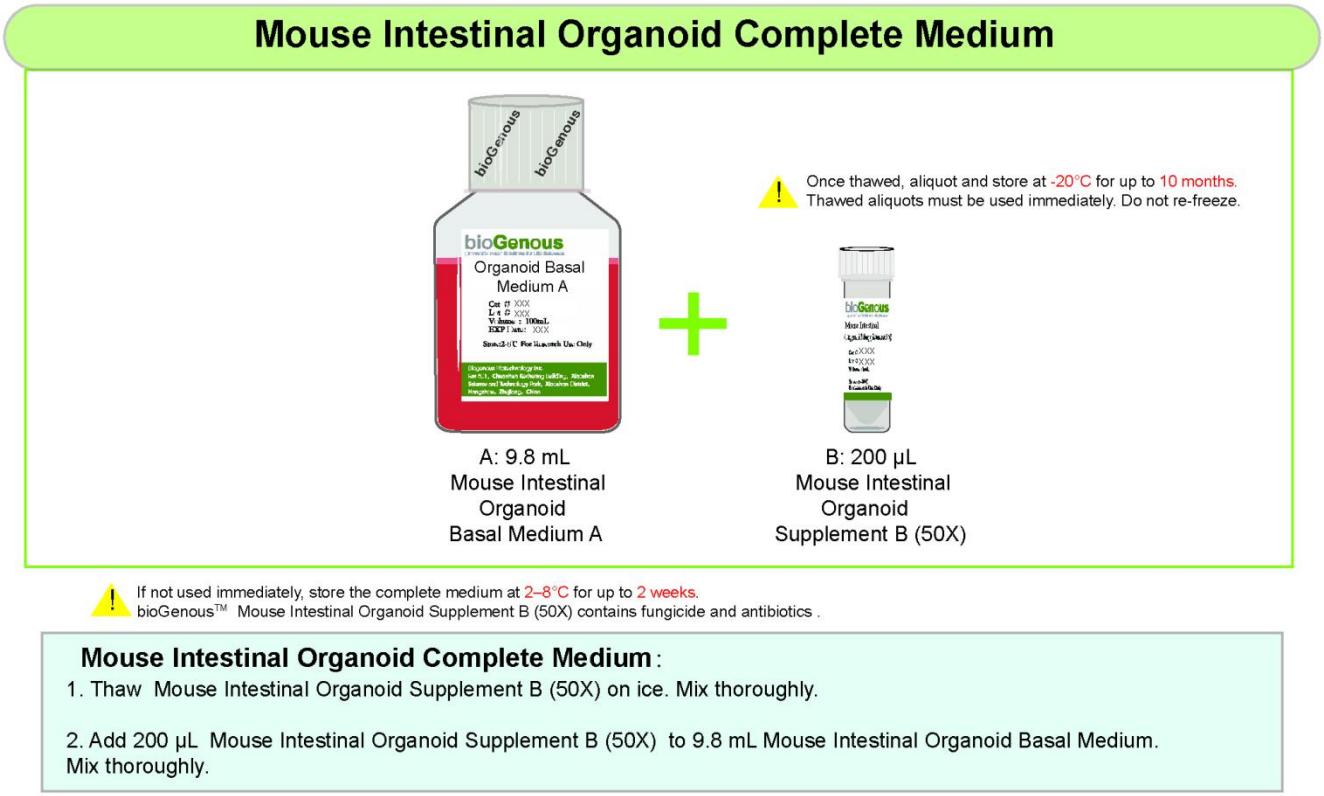
Preparation Before Use

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 bioGenous™ Mouse Intestinal Organoid Complete Medium (Basal Medium A + Supplement B). The following example is for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.



Mouse Intestinal Organoid Complete Medium:

1. Thaw Mouse Intestinal Organoid Supplement B (50X) on ice. Mix thoroughly.

2. Add 200 µL Mouse Intestinal Organoid Supplement B (50X) to 9.8 mL Mouse Intestinal Organoid Basal Medium. Mix thoroughly.

Protocol for Establishing Mouse Intestinal Organoids

Establishment of Organoids from Primary Tissue

1. Harvest mouse small intestine (3–20 cm in length) according to approved institutional guidelines and immediately place it in cold (2-8 °C) DPBS.
   NOTE: *Given potential variability between users, it is recommended to harvest a longer segment of small intestine during initial implementation to ensure adequate crypt recovery.*
2. Place the intestine in a 10 cm dish containing 5 mL of cold DPBS.
3. Flush the intestine gently with cold DPBS by inserting a 1 mL pipette tip into one open end of the intestine.
4. Use small scissors to make a longitudinal incision along the entire length of the intestine. Splay open the intestinal segment 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 villi and luminal contents. Transfer the tissue to a new dish containing cold DPBS and rinse thoroughly.
5. Cut the tissue into ~2 mm fragments and transfer them to 30 mL of pre-cooled DPBS containing 5 mM EDTA. Incubate at 4 °C for 30 min.
6. Following incubation, transfer the tissue fragments to a new culture dish containing DPBS and gently wash twice to remove EDTA.
7. Transfer the tissue fragments into 30 mL of cold Epithelial Organoid Basal Medium (B213151). Gently pipette up and down several times with a 5 mL pipette to resuspend the intestinal tissue fragments. When examination of a small aliquot under a brightfield microscope reveals abundant intestinal crypts, collect the supernatant and filter it through a 70 µm cell strainer into a 50 mL conical tube.
8. Centrifuge the fractions at 300 × g for 5 minutes at 4 °C. Carefully aspirate and discard the supernatant, leaving the pellet in the tube.
9. Resuspend the pellet in 1 mL cold DPBS. Remove 20 µL for microscopic counting of crypts. Then, centrifuge at 300 × g for 3 minutes at 4 °C. Discard the supernatant.

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 ECM on ice and complete the mixing within 30 seconds to prevent premature solidification.
    NOTE: *The final ECM concentration should be ≥ 70% (v/v) to ensure structural stability during culture.*
11. Plate 30 µL droplets of the ECM–crypt suspension into the center of each well of a 24-well plate, avoiding contact with the well walls.
    NOTE: *Perform this step rapidly to prevent ECM solidification at room temperature.*
12. Place the plate in a 37 °C, 5% CO_2 incubator for approximately 20 minutes.
13. After ECM solidification, gently add 500 µL complete medium per well.
    NOTE: *Add medium slowly along the wall of the well to avoid disrupting the ECM dome.*
14. Place the 24-well plate in a 37 °C, 5% CO_2 incubator for culture.
15. Change the medium every 3 days. Crypts from the small intestine typically start to bud after 2–4 days in culture.

Passaging of Organoids

1. Remove the culture medium from each well designated for passaging without disturbing the ECM dome containing the organoids.
2. Carefully detach the ECM gel from the well using 1 mL of Gentle Epithelial Organoid Basal Medium (B213151) and transfer it into a 1.5 mL centrifuge tube.
3. Using a pipette tip pre-rinsed with bioGenous™ Anti-Adherence Rinsing Solution (E238002), pipette up and down repeatedly to dissociate organoids from the ECM.
4. Centrifuge at 150 × g for 3 minutes at 4 °C. Carefully discard the supernatant and resuspend the pellet in DPBS. Centrifuge again at 150 × g for 3 minutes at 4 °C. Discard the supernatant and keep the pellet on ice.
5. Resuspend the pellet in an appropriate volume of ECM. Keep the ECM on ice and complete mixing within 30 seconds to prevent premature solidification.
   NOTE: *The final ECM concentration should be ≥ 70% (v/v) to ensure structural stability during culture.*
6. Plate 30 µL droplets of the ECM-organoid suspension into the center of each well of a 24-well plate, avoiding contact with the well walls.
   NOTE: *Perform this step rapidly to prevent ECM solidification at room temperature.*
7. Incubate the plate in a 37 °C, 5% CO_2 incubator for approximately 20 minutes.
8. After ECM solidification, gently add 500 µL of complete medium to each well.
9. Place the plate in a 37 °C, 5% CO_2 incubator for continued culture and downstream applications.

Organoid Passage Timing and Morphology

1. To maintain stable growth and optimal viability of mouse small intestinal organoids, passaging is recommended after approximately 5 days of culture or when organoids reach a diameter of 200–300 µm. Passaging should also be performed promptly when noticeable cell shedding appears within the lumen, when the organoid structure enlarges progressively, or when the organoids become dense in morphology, in order to prevent overgrowth that may compromise organoid viability and culture quality.
2. During the later stages of culture, black or dark regions may appear within the lumen of some organoids. These regions typically consist of cellular debris formed by the accumulation of senescent or shed cells and represent a common phenomenon in organoid culture, rather than indicating complete organoid death. If the organoids retain an intact structure and continue to grow, they can generally be considered to be in a normal and viable culture state.

   NOTE: *When passaging by dissociating into single cells, supplementary Wnt signaling components are required.*

Applications

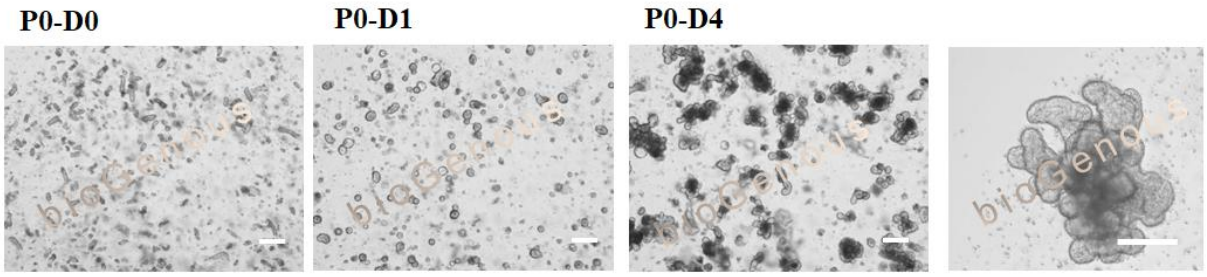


Figure 1. bioGenous™ Mouse Intestinal Organoid Prime Kit: Primary mouse small intestinal organoids at Day 0, 1, 4 and magnified view (10× objective) on Day 4. Scale bar: 250 μm.

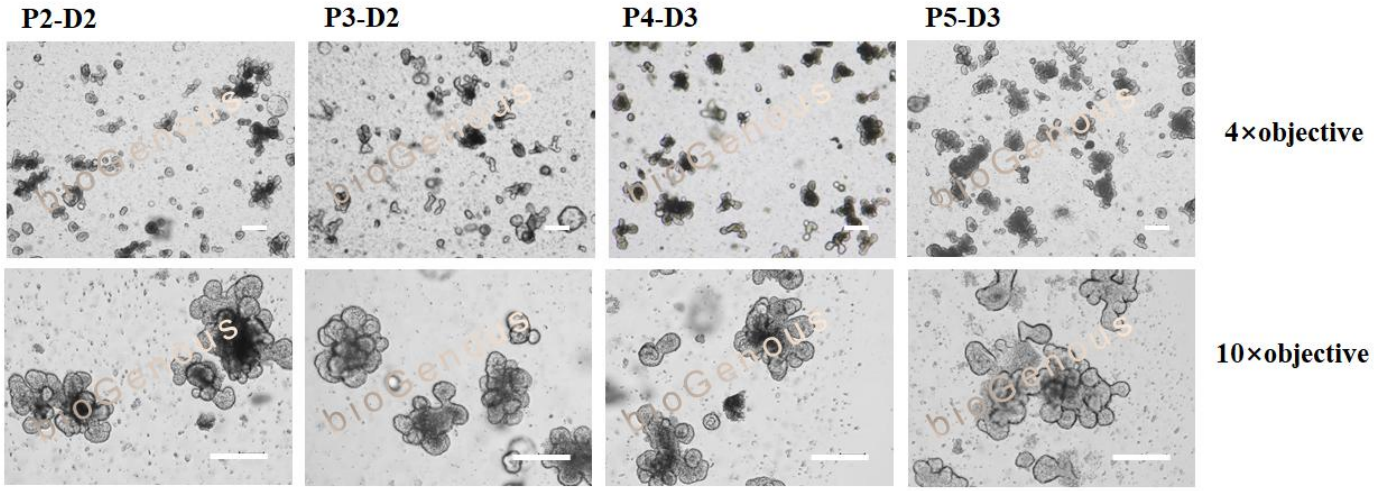


Figure 2. bioGenous™ Mouse Intestinal Organoid Prime Kit: Serially passaged mouse small intestinal organoids at Day 2, 3 and magnified view (10× objective) on Day 4. Scale bar: 250 μm.

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.

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