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 expansion 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 colonic biology.

Product Information

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Mouse Colonic Organoid Basal Medium A	K2204-MC-A100/A500	100 mL/500 mL	2-8°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/1 mL×2	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™	Primary Tissue Storage Solution	K601005
bioGenous™	Tissue Digestion Solution Plus	K601010
bioGenous™	Epithelial Organoid Basal Medium	B213151
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Organoid Cryopreservation Medium (Serum-free)	E238023
	DPBS (1X), liquid, contains no calcium or magnesium	-
	100 μm cell strainer	-
	Cell counting plate	-
	24-well cell culture plate	-
	Pipette and 0.2 mL, 1 mL, 5 mL pipette tips	-
	15 mL, 50 mL centrifuge tubes, 1.5 mL 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.
For research use only, not for use in diagnostic procedures.

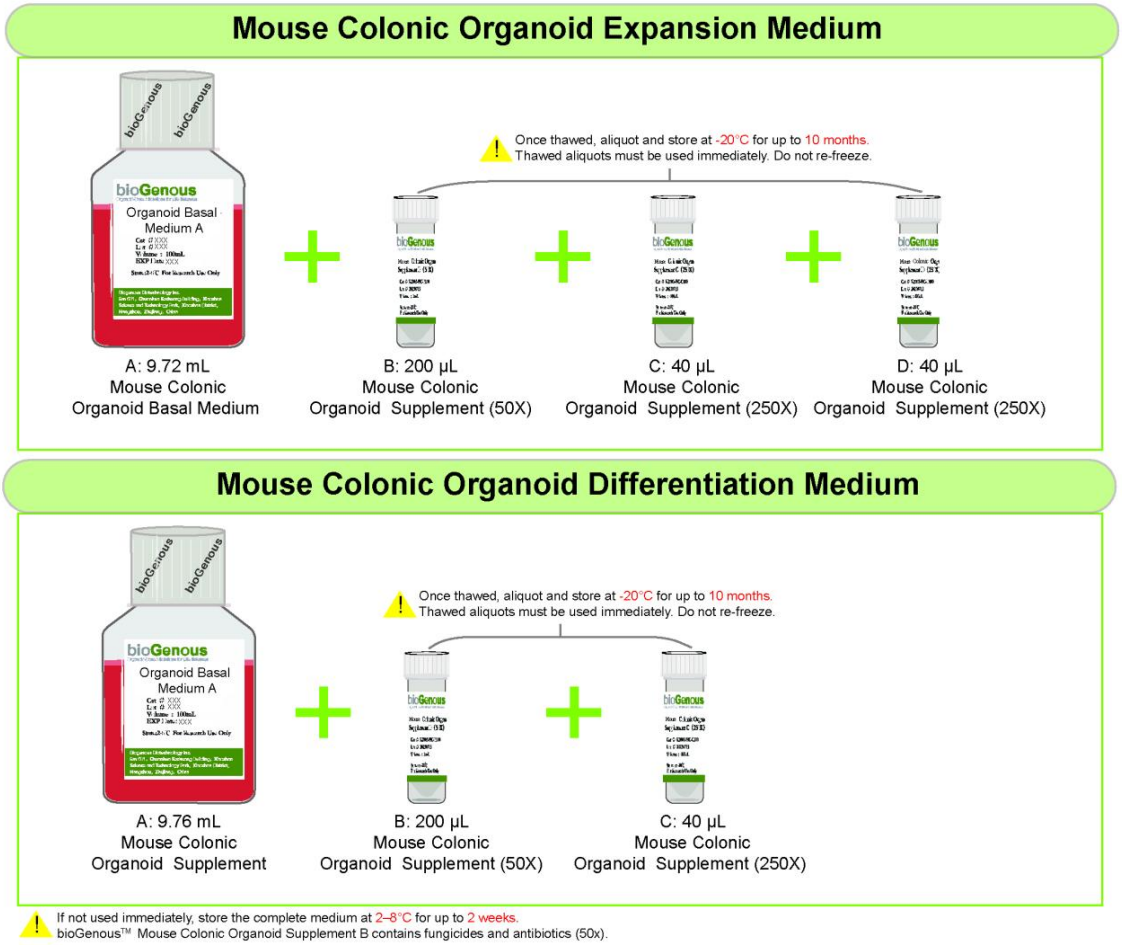
Preparation Before Use

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 Colonic Organoid Expansion Medium and Differentiation Medium

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



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

Mouse Colonic Organoid Expansion Medium and Differentiation 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 Expansion 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 Differentiation Medium** which used specifically for downstream functional studies.
Add 200 µL of Mouse Colonic Organoid Supplement B (50X) and 40 µL of Mouse Colonic Organoid Supplement C (250X) to 9.76 mL Mouse Colonic Organoid Basal Medium. Mix thoroughly.

4. The concentration and exposure time of supplement D are critical factors that influence the degree of organoid differentiation, with higher concentrations and longer exposure times typically resulting in reduced differentiation.
***Note**: This protocol can be adjusted, as the amount and duration of supplement D application may be modified according to the specific experimental conditions.*

Protocol for Establishing Mouse Colonic Organoids

⚠ Studies involving primary mouse tissue material must follow all relevant institutional and governmental regulations.

Establishment of Mouse Colonic Organoids from Primary Tissue

1. Collect mouse colonic tissue in accordance with institutional ethical and animal handling regulations. Immediately transfer the dissected tissue into bioGenous™ Primary Tissue Storage Solution (K601005) or ice-cold DPBS, and transport it promptly to a biosafety cabinet for subsequent processing.
2. Prepare several sterile culture dishes containing pre-chilled (4°C) DPBS supplemented with antibiotics.
3. Dissect a segment of distal colon approximately 3 cm from the anus, where fecal pellets are firm and well formed. Place the colon segment into a dish containing DPBS.
4. Carefully open the colon longitudinally using sterile scissors with the mucosal side facing upward. Rinse the lumen gently 4–5 times with antibiotic-containing DPBS to remove fecal residues.
5. Using a sterile scalpel blade or glass slide, gently scrape the mucosal surface to remove mucus and surface lipids. Transfer the cleaned tissue to a new dish containing fresh DPBS and wash once more.
6. This product recommends two methods for isolating colonic crypts, with the bioGenous™ Tissue Digestion Solution Plus being the recommended approach. This product recommends two methods for isolating crypt epithelium of the colon, among which bioGenous™Tissue Digestion Solution Plus is the preferred approach.
   a. **Tissue Digestion Solution Plus-based method:** Cut the cleaned tissue into small fragments of approximately 5 mm in length and transfer them into a 15 mL centrifuge tube containing 15 mL of pre-warmed (37°C) bioGenous™ Tissue Digestion Solution Plus (K601010).
   b. Place the tube into the bioGenous™ SmartOrgan™ Dissociator and digest at 37°C for 15 min.
   c. Alternatively, digestion can be performed in a 37°C incubator for 15-30 min.
      ***NOTE**: The total digestion time should not exceed 30 minutes to preserve crypt viability.*
   d. Every 5 minutes, gently pipette up and down approximately 10 times using a cut 1 mL pipette tip to facilitate crypt release. Withdraw a small aliquot of the digestion mixture and observe under a microscope. When a large number of colonic crypt epithelium become clearly visible, immediately terminate the digestion by adding FBS to a final concentration of 2%, and mix gently.
   e. Cut off the end of a 1 mL pipette tip and gently pipette up and down several times to release additional crypts.
   f. **EDTA-based method:** Mince colonic segments into approximately 2 - 5 mm (in length and width) pieces and transfer them to 2 mM EDTA in DPBS. Incubate at 4°C for 30 min. (At a ratio of 10 mL EDTA solution per 1 cm of colonic segment length)
   g. After incubation, transfer the tissue pieces to a dish containing fresh DPBS and wash thoroughly to completely remove any residual EDTA.
   h. Resuspend the tissue pieces in cold DPBS or Epithelial Organoid Basal Medium and mechanically dissociate them by pipetting up and down through a 5 mL pipette to release crypts.
7. Filter the digested suspension through a 100 μm cell strainer, and rinse the filter with approximately 5 mL of bioGenous™ Epithelial Organoid Basal Medium (B213151) to collect the remaining crypts.
8. Collect the filtrate and centrifuge at 300 × g for 3 minutes at 4°C. Carefully discard the supernatant, resuspend the pellet in cold basal medium, and centrifuge once again to remove residual enzymes. Keep the crypt pellet on ice for immediate embedding.
9. Resuspend the crypt pellet in pre-cooled bioGenous™ Organoid Culture ECM (M315066). The recommended density is 50–300 crypts per 25 μL ECM. Gently mix to achieve a homogeneous suspension, avoiding air bubbles. Keep the suspension on ice and limit resuspension time to within 30 seconds to prevent premature ECM polymerization.
   ***NOTE**: ECM concentration should be ≥70% (v/v) to maintain 3D structure during culture.*
10. Carefully dispense 25 μL of the ECM–crypt mixture into the center of each well of a 24-well plate, avoiding contact with the well wall.
    ***NOTE**: Perform this step rapidly to prevent ECM solidification at room temperature.*
11. Place the plate in a 37°C, 5% CO_2 incubator for approximately 20 minutes to allow ECM polymerization.
12. After ECM solidification, gently add 500 μL of pre-warmed Mouse Colonic Organoid Expansion Medium along the sidewall of each well. Add sterile water to the peripheral wells of the 24-well plate to maintain humidity.
    ***CAUTION**: Add the medium slowly along the wall to avoid disturbing the ECM dome.*

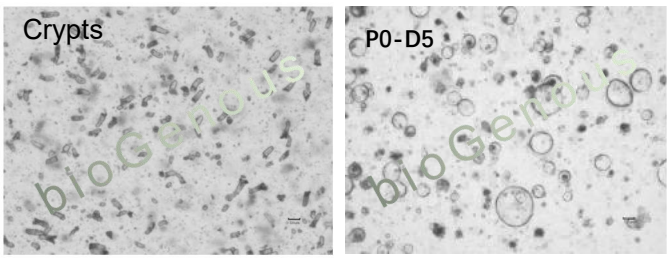
13. Incubate the plate at 37°C, 5% CO_2. Replace the medium every 3 days, ensuring that the ECM structure remains intact during medium changes.
14. Under optimal conditions, primary mouse colonic organoids will form within 5–7 days after embedding.

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 bioGenous™ 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 expansion medium at 37°C.
   Note: *For downstream functional studies, the culture medium can be transitioned to differentiation medium immediately following passaging. If continued expansion is desired, maintain the organoids in expansion medium under standard culture conditions.*
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. Finally, we will produce organoids that faithfully recapitulate the complete cellular composition and function of native colonic epithelium.

Applications

Tissue Digestion Solution Plus-based digestion method



EDTA-based digestion method

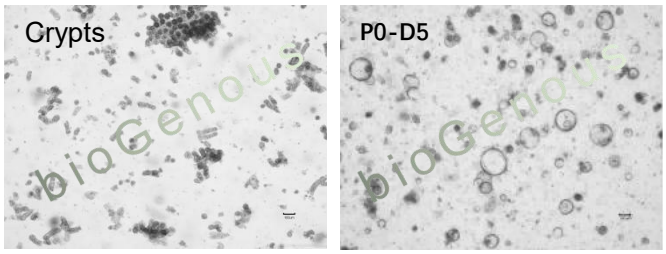


Figure 1. Comparison of crypt isolation efficiency and organoid formation between the Tissue Digestion Solution Plus method and the EDTA-based digestion method. The Tissue Digestion Solution method yields a higher number of intact crypts with preserved basement structure, whereas the EDTA method produces fewer crypts. Scale bar: 100 μm.

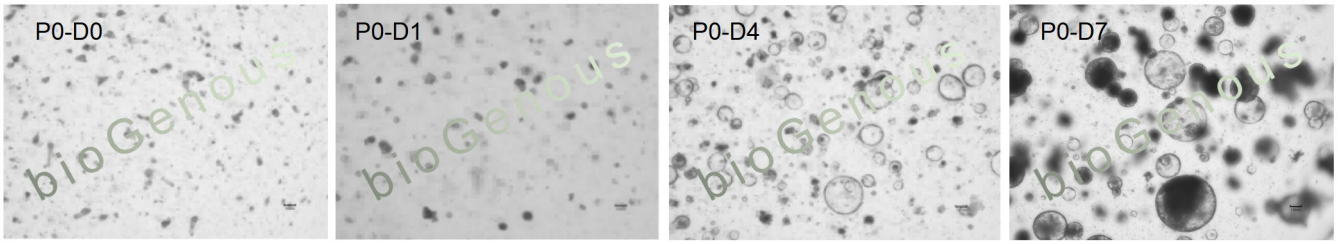


Figure 2. Morphological examples of primary culture of mouse colonic organoid in expansion medium. Passage number, as well as days post embedding in the passage, are indicated above each image. Scale bar: 100 μm.

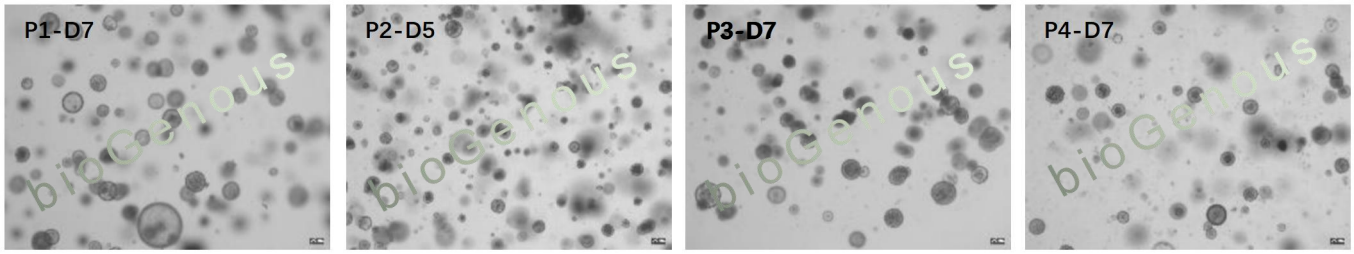


Figure 3. Representative images of mouse colonic organoid across different passages in differentiation medium. Passage number, as well as days post embedding in the passage, are indicated above each image. Scale bar: 100 μm.

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.

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