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	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™	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
bioGenous™	Primary Tissue Storage Solution(Serum-free)	K601005
	DPBS (1x), liquid, contains no calcium or magnesium	-
	70 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

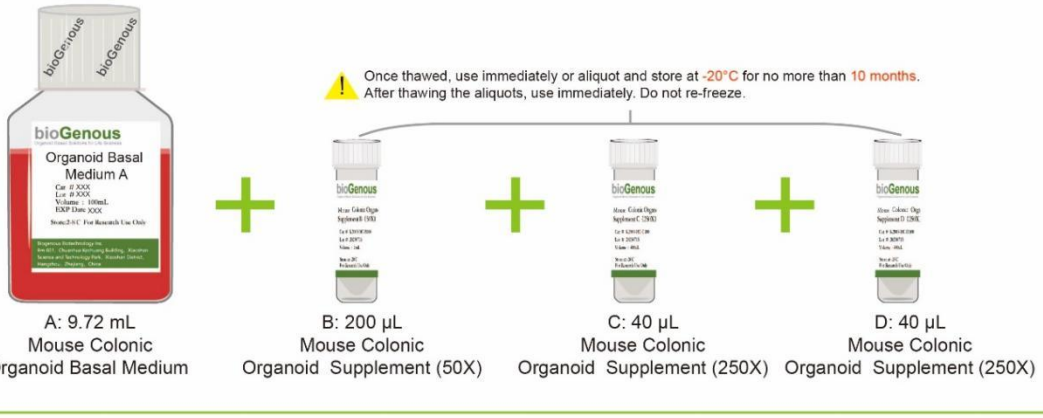
Before initiating the protocol, ensure that all components and equipment are properly prepared:

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.

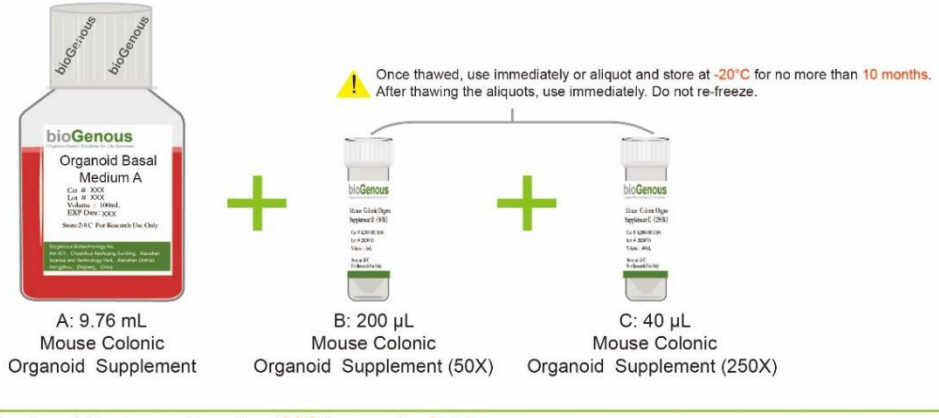
Preparation of Mouse Colonic Organoid Expansion Medium and Differentiation Medium

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.

Mouse Colonic Organoid Expansion Medium



Mouse Colonic Organoid Differentiation Medium



If not use immediately, store complete medium at 2-8°C for no more than 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 colon tissue in accordance with institutional guidelines for animal research ethics and aseptic surgical procedures. Immediately transfer tissue samples to a clean laboratory using Primary Tissue Storage Solution (K601005) or DPBS at 2–8°C.
2. Prepare several culture dishes containing pre-chilled (4°C) DPBS.
3. Dissect a segment of mouse colon (3-10 cm depending on experimental needs) and place it into a dish containing DPBS.

4. In a biosafety cabinet, use a pipette to gently flush one end of the colon with DPBS to remove fecal content. Repeat several times until the lumen is visibly clean. Transfer the tissue to a new dish with fresh DPBS.
5. Use surgical scissors to cut the colon longitudinally with the mucosal side facing upward. Hold one end with forceps and gently scrape the mucosal surface using a sterile scalpel to remove the top mucous layer. Transfer the cleaned tissue to a new dish containing DPBS and wash again once more.
6. Mince the tissue into ~2 mm pieces and transfer to 5–10 mL of ice-cold DPBS containing 2 mM EDTA, incubate at 4°C for 30 min.
7. After incubation, transfer the tissue fragments to a new dish with fresh DPBS and wash again to remove residual EDTA.
8. Resuspend the tissue fragments in cold DPBS and mechanically dissociate them by pipetting up and down through a 5 mL pipette to release crypts.
   Note: *Monitor under a microscope. Once a large number of crypt-like structures are observed, stop pipetting and filter the suspension through a 70 μm cell strainer.*
9. Collect the flow-through and centrifuge at 300 x g, 4°C for 3 min.
10. Discard the supernatant and resuspend the pellet in 1 mL Epithelial Organoid Basal Medium (B213151). Take 20 μL for crypt counting under the microscope. Based on the count, transfer the appropriate volume containing the desired number of crypts, centrifuge at 300 x g, 4°C for 3 min, discard the supernatant, and keep the pellet on ice.
11. Resuspend the pellet in appropriate volume of organoid culture ECM (M315066), recommended at 50-300 crypts per 25 μL ECM. Keep on ice and minimize resuspension time to under 30 seconds to avoid premature gelation.

    Note: *ECM concentration should be ≥70% (v/v) to maintain 3D structure during culture.*

12. Gently mix ECM-crypts, avoiding bubbles. Dispense 25 μL per well to the center of each well in a 24-well plate. Avoid contact with the sidewalls.
    Note: *Perform quickly to avoid ECM solidifying at room temperature.*
13. Place the plate in a 37°C CO_2 incubator for 20 min to allow ECM polymerization.
14. Prewarm the mouse colonioc organoid primary medium.
15. After ECM solidification, gently add 500 μL of prewarmed expansion medium along the sidewall of each well. Add 500 μL sterile water to outer well of the 24-well plate to maintain humidity. Incubate at 37°C, 5% CO_2.
    Note: *Add media along the wall slowly to avoid disturbing the ECM.*
16. Change medium every 3 days, and avoid damaging ECM during medium changes. Primary mouse colonic organoids should form within 5-7 days.

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 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 obtain organoids that perfectly replicate in vivo properties of colonic epithelial cell composition and function.

Applications

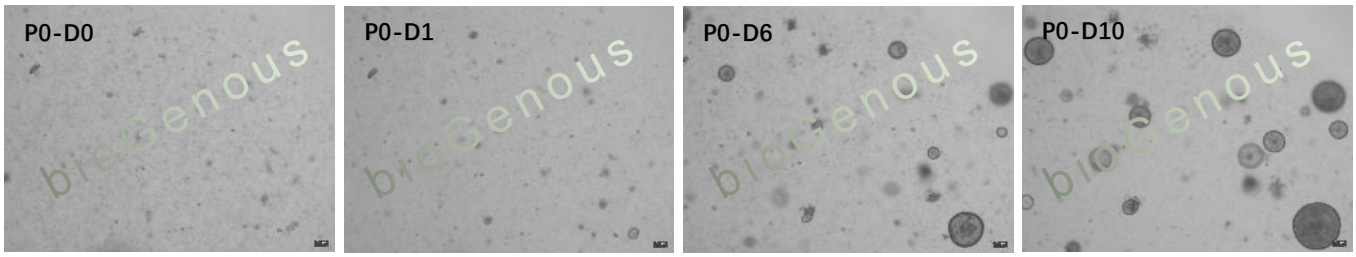


Figure 1. Morphological examples of primary culture of mouse colonic organoid in expansion medium. Passage number, as well as days post embedding in the passage, are indicated below each image. Scale bar: 100 μm.

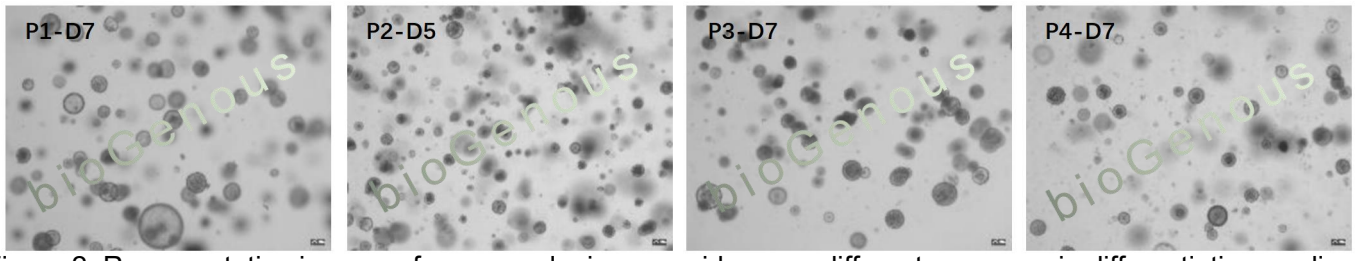


Figure 2. Representative images of mouse colonic organoid across different passages in differentiation medium. Passage number, as well as days post embedding in the passage, are indicated below each image. Scale bar: 100 μm.

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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