bioGenous™ Human Colonic Organoid Kit (Serum-free)

Catalog: K2003-HC

Product Description

bioGenous™ Human Colonic Organoid Kit is a serum-free culture medium for human colonic organoids derived from adult stem cells. Self-renewal of colonic epithelium is driven by the proliferation of stem cells and their progenitors located in the crypts. Human colonic organoids grown in primary culture medium consisted of LGR5+ stem cells, cycling transit amplifying (TA) cells, early enterocytes (CDX2+, Villi+). While human colonic organoids grown in maintenance medium contain mature enterocytes, goblet cells (MUC2+), M cells (GP2+) and enteroendocrine cells, as well as a low number of tuft cells. Human colonic organoids display hallmarks of the colonic epithelium in terms of architecture and cell type composition, therefore hold great promise for studies of human colonic development and disease, human colonic organoids may also have applications in regenerative biology through ex vivo primary culture of the colonic epithelium.

Product Information

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Human Colonic Organoid Basal Medium	K2003-HC-A100/A500	100 mL/500 mL	4°C, 12 months
bioGenous™ Human Colonic Organoid Supplement B (50x)	K2003-HC-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Colonic Organoid Supplement C (250x)	K2003-HC-C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Colonic Organoid Supplement D (250x)	K2003-HC-D100/D500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 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™	Primary Tissue Storage Solution (Serum-free)	K601005
bioGenous™	Epithelial Organoid Basal Medium (Serum-free)	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Anti-Adherence Rinsing Solution	E238002
bioGenous™	Organoid Cryopreservation Medium (Serum-free)	E238023
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
	Fetal Bovine Serum (FBS)	-
	DPBS (1x), liquid, contains no calcium or magnesium	-
	100 μm cell strainer	-

Preparation Before Use

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

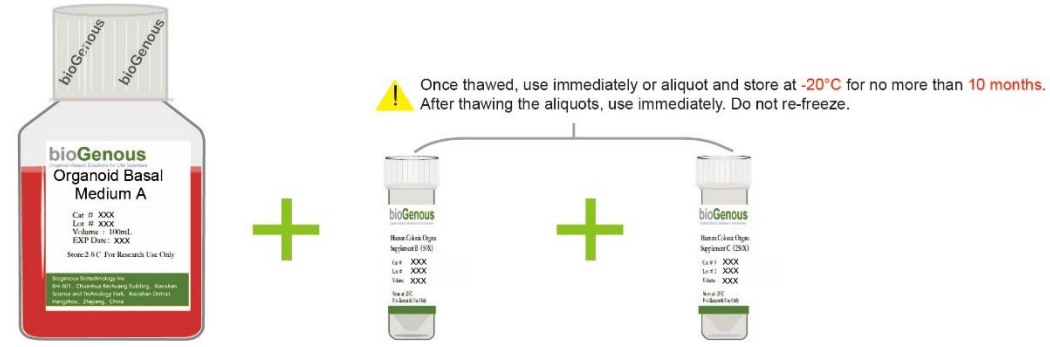
1. Verify that all components are stored according to the guidelines provided in the manual. Avoid repeated freeze-thaw cycles for sensitive reagents. Thaw all necessary reagents according to the instructions. Keep on ice or at the recommended temperature until ready to use.
2. Ensure that all equipment,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

Preparation of Human Colonic Organoid Primary Culture Medium and Maintenance Medium

Use a sterile technique to prepare the human colonic organoid primary culture medium and maintenance medium. The following examples are for preparing 10 mL of primary culture medium and maintenance medium. If preparing other volumes, adjust accordingly.

Human Colonic Organoid Primary Culture Medium

Human Colonic Organoid Maintenance Medium



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

Human Colonic Organoid Primary Culture Medium and Maintenance Medium:

1. Thaw Human Colonic Organoid Supplement B (50x), Human Colonic Organoid Supplement C (250x) and Human Colonic Organoid Supplement D (250x) on ice. Mix thoroughly.

2. For **Human Colonic Organoid Primary Culture Medium** which used specifically for primary culture and resuscitation.
Add 200 µL Human Colonic Organoid Supplement B (50x), 40 µL Human Colonic Organoid Supplement C (250x) and 40 µL Human Colonic Organoid Supplement D (250x) to 9.72 mL Human Colonic Organoid Basal Medium.
Mix thoroughly.

3. For **Human Colonic Organoid Maintenance Medium** which used specifically for long-term culture.
Add 200 µL of Human Colonic Organoid Supplement B (50x) and 40 µL of Human Colonic Organoid Supplement C (250x) to 9.76 mL of Human 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 Establishment of Human Colonic Organoids

⚠ Studies involving primary human tissue material must follow all relevant institutional and governmental regulations. Informed consent must be obtained from all subjects before the collection of the primary human tissue material.

Establishment of Organoids from Primary Tissue

1. Collect primary human colonic tissue biopsies in ice-cold Primary Tissue Storage Solution (K601005) with conical tubes. Keep tissue biopsies at 4°C until the start of the isolation.
2. Rinse the tissue with Epithelial Organoid Basal Medium (B213151) until the supernatant is clear.
3. Thaw bioGenous™ Organoid Culture ECM (M315066) on ice and keep it cold.
4. Mince the tissue into small fragments in a cell culture dish using surgical scissors or scalpels.
   CRITICAL *The minced samples must be small enough to pass through the tip of a 1 mL pipette.*
5. Digest the tissue fragments by adding 10 mL of Tissue Digestion Solution (K601003) in a 15 mL conical tube at 37°C, with variable incubation times ranging from 30 min to 1 h. Carefully monitor the digestion process, mixing the content of the tube every 5-10 min by shaking vigorously or pipetting the mixture up and down.
   CRITICAL *To avoid over-digestion, stop when numerous cell clusters appear under the microscope.*
6. Terminate tissue digestion by adding FBS to the tissue digestion mixture to a final concentration of 2% and filter through a 100 µm cell strainer.
7. Collect and centrifuge the filtered cells at 250 x *g* for 3 min at 4°C. In case of a visible red pellet, aspirate the supernatant, and resuspend the pellet using 1 mL of Red Blood Cell Lysis Solution (E238010) to lyse the erythrocytes at room temperature for 3 min and centrifuge at 250 x *g* for 3 min at 4°C.
8. Aspirate the supernatant and resuspend the pellet in Epithelial Organoid Basal Medium, centrifuge at 250 x *g* for 3 min at 4°C. Repeat this step one more time.
9. Aspirate the supernatant and resuspend the pellet in ECM. ECM should be kept on ice to prevent it from solidifying; thus, work quickly. The amount of ECM depends on the size of the pellet. Approximately 10,000 cells should be plated in 25 µL of ECM.
   CRITICAL *Do not overly dilute the ECM (>70% (ECM vol/total vol)), as this may inhibit the proper formation of the solid droplets*.
10. Seed the ECM containing cells at the bottom of 24-well cell culture plates in droplets of ~30 µL each around the center of the well.
    CRITICAL *Once the cells are resuspended in ECM, proceed with plating as quickly as possible, as the ECM may solidify in the tube or pipette tip. Do not let the ECM touch the walls of well.*
11. Place the culture plate into a humidified incubator at 37°C and 5% (vol/vol) CO_2 for 15-25 min to let the ECM solidify.
12. Prepare the required amount of bioGenous™ human colonic organoid primary culture medium.
13. Once the ECM droplets are solidified (15-25 min), open the plate and carefully add 500 µL organoid primary culture medium to each well.
    CRITICAL *Do not add the medium directly on top of the ECM droplets, as this might disrupt the droplets*.
14. Place the culture plate in a humidified incubator at 37°C and 5% (vol/vol) CO_2.
15. Change the medium every 3 days by carefully aspirating the medium from the wells and replacing it with fresh, pre-warmed human colonic organoid primary culture medium.
16. Closely monitor organoid formation. Ideally, human colonic organoids should be passaged for the first time between 5 and 8 days after initial seeding. Typical morphologies of successfully cultured human colonic organoid are shown in Figure 1.

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 human colonic organoid maintenance medium at 37°C.
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.

Applications

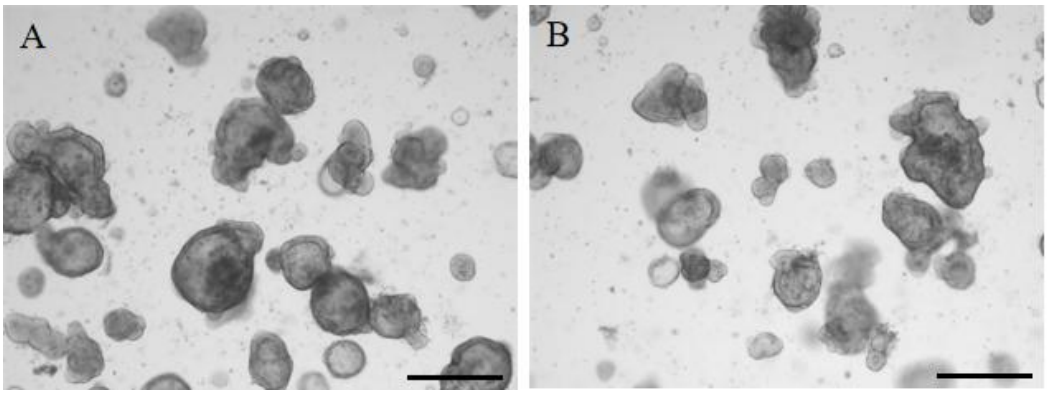


Figure 1. Examples of successful culture in primary culture medium at day 7, and in maintenance medium at day 5 of human colonic organoids. Scale bar: 200 μm.

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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