bioGenous™ Colorectal Cancer Organoid Kit

Catalog: K2103-CR

Product Description:

bioGenous™ Colorectal Cancer Organoid Kit is a chemically defined cell culture medium for the human Colorectal cancer organoids. Patient-derived cancer organoids recapitulate the genomic and pathological features of original tumors and therefore hold great promise for medical research and precision medicine.

Product Information:

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Colorectal Cancer Organoid Basal Medium	K2103-CR-A100/A500	100mL/500mL	2-8℃，12 months
bioGenous™ Colorectal Cancer Organoid Supplement B (50x)	K2103-CR-B100/B500	2mL/10mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Colorectal Cancer Organoid Supplement C (250x)	K2103-CR-C100/C500	0.4mL/2mL	-20℃, avoid repeated freeze-thaw cycles, 12 months

Materials & Reagents Required But Not Included:

Manufacturer	Materials	Catalog#
bioGenous™	Primary Tissue Storage Solution	K601005
bioGenous™	Cancer Organoid Basal Medium	B213152
bioGenous™	Tumor Tissue Digestion Solution	K601003
bioGenous™	Red Blood Cell Lysis Solution	E238010
bioGenous™	Anti-Adherence Rinsing Kit	E238002
bioGenous™	Organoid Cryopreservation Medium(Serum Free)	E238023
bioGenous™	Organoid Dissociation Solution	E238001
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 of Colorectal Cancer Organoid Complete Medium

Use a sterile technique to prepare the colorectal cancer organoid complete medium. The following example is for preparing a 10mL complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Colorectal Cancer Organoid Supplement B (50x) and Colorectal Cancer Organoid Supplement C (250x) on ice. Mix thoroughly.
   NOTE: Once thawed, use immediately or aliquot and store at -20°C for not more than 10 months. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 200μL Colorectal Cancer Organoid Supplement B (50x) and 40μL Colorectal Cancer Organoid Supplement C(250x) to 9.76mL Colorectal Cancer Organoid Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store the complete medium at 2-8°C for not more than 2 weeks. The Colorectal Cancer Organoid Supplement B contains fungicides and antibiotics (50x).

Protocol for Establishment of Patient-Derived Colorectal Cancer Organoids

CAUTION *Studies involving primary human tissue material must follow all relevant institutional and government 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 colorectal cancer tissue pieces in ice-cold Primary Tissue Storage Solution (K601005) with conical tubes. Keep tissue samples at 4°C until the start of the isolation.
2. Assess whether the obtained tissue pieces consist purely of epithelium. If fat or muscle tissues are present, remove these non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. If no fat or muscle tissues are present, continue to the next step immediately.
3. Rinse the tissue with Epithelial Organoid Basal Medium (B213152) until the supernatant is clear.
4. Thaw bioGenous™ Organoid Culture ECM (M315066) on ice and keep it cold.
5. Mince the tissue into small fragments in a cell culture dish using surgical scissors or scalpels.
   CRITICAL The dissected samples must be small enough to pass through the tip of a 10 mL pipette.
   Digest the tissue fragments with 10mL of Tissue Digestion Solution(K601003) in a 15mL conical tube at 37°C, with

a variable incubation period ranging from 20 min to 30 min Carefully monitor the digestion process by mixing the content of the tube every 5-10 min by shaking vigorously and pipetting the mixture up and down.
CRITICAL To prevent over-digestion, one should examine the cells under the microscope if the epithelium cell clusters appear during digestion.

6. Add FBS to the tissue digestion mixture to a final concentration of 2% and filter using a 100 μm cell strainer.
7. Collect and centrifuge the filtered cells at 250g for 3 min at 4 °C. In case of a visible red pellet, aspirate the supernatant, and resuspend the pellet using 2mL of Red Blood Cell Lysis Solution (E238010) to lyse the erythrocytes at room temperature for 3 min and centrifuge at 250g for 3 min at 4°C.
8. Aspirate the supernatant and resuspend the pellet in Epithelial Organoid Basal Medium, centrifuge at 250g for 3 min at 4°C, and 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 20-100 ducts should be plated in 25 μL of ECM.
   CRITICAL Do not dilute ECM too much (ECM should be >70% (ECM vol/Total vol)) to ensure the formation of solid droplets.
10. Plate the ECM containing organoids on the bottom of 24-well cell culture plates in droplets of ~30 μL each around the center of the well.
    CRITICAL Once the organoids 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 wall of the wells.
11. Place the culture plate into a humidified incubator at 37 °C and 5% (vol/vol) CO2 for 15-25 min to let the ECM solidify.
12. Prepare the required amount of bioGenous™ Human Colorectal Cancer Organoid Medium.
13. Once the ECM droplets are solidified (15-25 min), open the plate and carefully add 500 μL of Organoid Complete 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 Colorectal Cancer Organoid Medium.
16. Closely monitor organoid formation. Ideally, human Colorectal Cancer organoids should be passaged for the first time between 5 and 8 days after initial plating. Typical examples of the various morphologies of successfully cultured human colorectal cancer organoids are shown in Figure 1 and examples of successful culture in primary, passage and resuscitation of human colorectal cancer organoids are shown in Figure 2.

Splitting and Passaging of Organoids

17. Pipette up and down to disrupt the ECM and transfer the organoid suspension to a 1.5 mL conical tube.
18. Pipette the organoid suspension up and down to mix thoroughly by pipetting against the bottom of the tube to create pressure, which will aid the removal of ECM.
19. Centrifuge organoids at 250g for 3 min at room temperature.
20. Aspirate the supernatant and split organoids using either Organoid Dissociation Solution (E238001) or by mechanical disruption. For 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 organoids fall apart. 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. In case of mechanical disruption, resuspend the pellet in 1.5 mL of Cancer 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 >6 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.
21. After shearing is complete, wash once by topping up with 1 mL of Cancer Organoid Basal Medium, and centrifuge at 250g for 3 min at room temperature.
22. 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 as described in Step 10. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15–25 min.
23. Pre-warm colorectal cancer organoid complete medium at 37 °C.
24. After the ECM droplets have solidified (15–25 min), carefully pipette the pre-warmed medium into the wells.
25. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO_2 until the organoids are needed for further experiments.

Appendix 1. Typical examples of the morphologies of human colorectal cancer organoids.

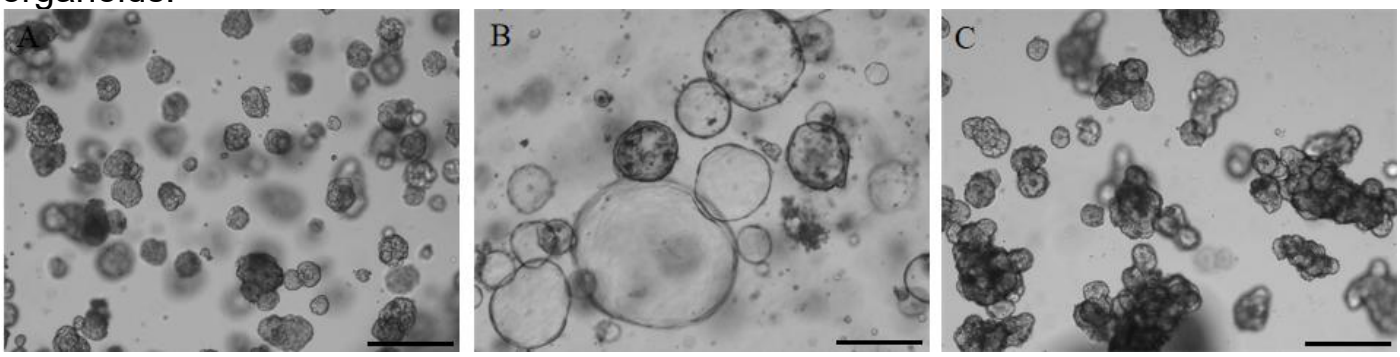

Figure 1. Morphological examples of successfully cultured colorectal cancer organoids derived from different patients. (A)The organoids showed two forms of vesicles or parenchyma with glandular cavity structure, with a diameter of about 50μm, and the growth trend of colon cancer organoids was good. (B)The organoids are mainly represented by vesicles, organoids show obvious cavities with a diameter of more than 200 μm. (C)The organoids showed irregular folds with smooth edges and high dioptering, mainly presented parenchymal shapes, and the diameter could reach more than 200μm.(scale bar: 200 μm).

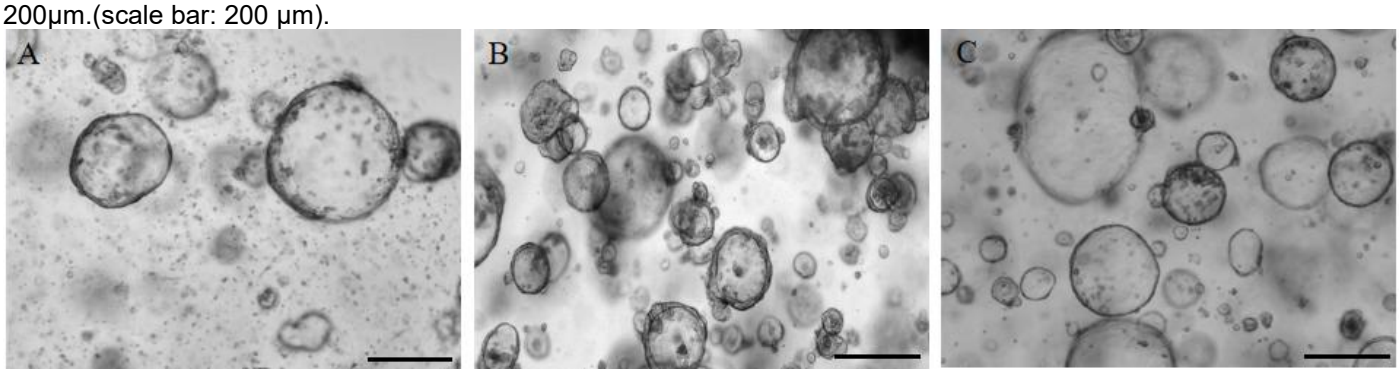

Figure 2. Examples of successful culture in primary, passage and resuscitation of human colorectal cancer organoids. (A) The growth status of colorectal cancer organoids in primary culture (P0), organoids mainly existed in vesicular form with a diameter of up to 200μm. (B) The growth status of colorectal cancer organoids in the first passage culture (P1), passage organoids show vesicle. (C) The resuscitated cultured organoids showed a steady growth trend,organoids show obvious cavities with a diameter of more than 200 μm.(scale bar: 200 μm).

Last updated on 29th July 2023