bioGenous™ Human Intestinal Organoid Kit

Catalog: K2002-HI

Product Description:

bioGenous™ Human Intestinal Organoid Kit is a chemically defined cell culture medium for human intestinal organoids(hIOs) derived from adult stem cells. Self-renewal of the intestinal epithelium is driven by the proliferation of stem cells and their progenitors located in the crypts. Human intestinal organoids display all hallmarks of the intestinal epithelium in terms of architecture, cell type composition, and self-renewal dynamics, therefore hold great promise for unprecedented studies of human intestinal development and disease, human intestinal organoids may also have applications in regenerative biology through ex vivo expansion of the intestinal epithelium.

Product Information:

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Human Intestinal Organoid Basal Medium	K2002-HI-A100/A500	100 mL/500 mL	4℃，12 months
bioGenous™ Human Intestinal Organoid Supplement B(50x)	K2002-HI-B100/B500	2 mL/10 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Intestinal Organoid Supplement C(250x)	K2002-HI-C100/C500	0.4 mL/2 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Intestinal Organoid Supplement D(250x)	K2002-HI-D100/D500	0.4 mL/2 mL	-20℃, avoid repeated freeze-thaw cycles, 12 months

Materials & Reagents Required But Not Included:

Manufacturer	Materials	Catalog#
bioGenous™	Primary Tissue Storage Solution	K601005
bioGenous™	Epithelial Organoid Basal Medium	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Anti-Adherence Rinsing Kit	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 of Human Intestinal Organoid Expansion Medium and Maintenance Medium

Use sterile technique to prepare the human intestinal organoid expansion medium and maintenance medium. hIOs grown in Human Intestinal Organoid Expansion Medium overwhelmingly consisted of $LGR5^+$ stem cells, cycling transit amplifying (TA) cells, early enterocytes and a small number of goblet cells. Organoids grown in Human Intestinal Organoid Maintenance Medium contain $LGR5^+$ stem cells, TA cells, early and mature enterocytes, goblet cells, M cells and enteroendocrine cells, as well as a low number of Paneth cells and tuft cells. The following examples are for preparing 10 mL of Expansion Medium and Maintenance Medium. If preparing other volumes, adjust accordingly.

1. Thaw Human Intestinal Organoid Supplement B (50x), Human Intestinal Organoid Supplement C (250x) and Human Intestinal Organoid Supplement D (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. For Human Intestinal Organoid Expansion Medium which used specifically for primary culture and resuscitation. Add 200 μL Human Intestinal Organoid Supplement B (50x), 40 μL Human Intestinal Organoid Supplement C (250x) and 40 μL Human Intestinal Organoid Supplement D (250x) to 9.72 mL Human Intestinal Organoid Basal Medium. Mix thoroughly.
3. For Human Intestinal Organoid Maintenance Medium which used specifically for transfer of culture. Add 200 μL Human Intestinal Organoid Supplement B (50x) and 40 μL Human Intestinal Organoid Supplement C (250x) to 9.76 mL Human Intestinal Organoid Basal Medium. Mix thoroughly.
   NOTE: If not use immediately, store complete medium at 2-8°C for not more than 2 weeks. bioGenous™ Human Intestinal Organoid Supplement B contains fungicides and antibiotics (50x).

Protocol for Establishment of Human Intestinal Organoids

CAUTION *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 intestinal 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 or if they also contain fat or muscle tissue. If so, remove non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. If no fat or muscle tissue are present, continue to the next step immediately.
3. Rinse the intestinal tissue with Epithelial Organoid Basal Medium(B213151) or DPBS until the supernatant is clear.
4. Before crypt isolation, thaw bioGenous™ Organoid Culture ECM on ice and keep it cold. Add 5 mL of FBS to 45 mL of Epithelial Organoid Basal Medium to prepare 10% (vol/vol) FBS medium.
5. Mince the tissue into small fragments of 5 mm^3 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.
6. Place the dissected pieces of sample into a 15 mL conical tube containing 10 mL of cold Epithelial Organoid Basal Medium with 1% FBS.
7. Wash the samples by pipetting up and down with a 10 mL pipette at least ten times.
   CRITICAL For the subsequent steps, coat the inner surface of pipette tips with bioGenous™ Anti-Adherence Rinsing Solution (E238002) before use to avoid the adherence of the samples on the pipette wall.
8. Stand the tube still until the samples settle at the bottom. Aspirate the supernatant with a 10 mL pipette and add 10 mL of pre-warmed Tissue Digestion Solution (K601008).
9. Digest the tissue fractions at 37℃ with rotation at the speed of 100 rpm. The digestion time should not exceed 30 mins.
   CRITICAL To prevent over-digestion, one should examine under the microscope if the duct structure appears during digestion.
10. Once the duct structure appears, stop digestion by the addition of FBS to a final concentration of 2% and pipette gently up and down.
11. Stand the tube for 1-2 min. Transfer the supernatant into a new tube.
12. Add 10mL Epithelial Organoid Basal Medium and repeat Step 11 one more time.
13. Spin the supernatant at 300g for 3 min at 4°C. Aspirate and discard the supernatant.
14. Re-suspend the pellet with 10mL Epithelial Organoid Basal Medium and spin at 300g for 3 min at 4°C.
15. Repeat Step 14 twice.
16. 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 cryptshould 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.
17. 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.
18. 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.
19. Prepare the required amount of bioGenous™ Human Intestinal Organoid Medium.
20. 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.
21. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO_2.
22. Change the medium every 3 days by carefully aspirating the medium from the wells and replacing it with fresh, pre-warmed Human Intestinal Organoid Medium.
23. Closely monitor organoid formation. Ideally, human Intestinal organoids should be passaged for the first time between 5 and 8 days after initial plating.Examples of successful culture in primary, passage and resuscitation of human intestinal organoids are shown in Figure 1.

Splitting and Passaging of Organoids

24. Pipette up and down to disrupt the ECM, and transfer the organoid suspension into a 1.5 mL conical tube.
25. Pipette the organoid suspension up and down to mix thoroughly. Use the bottom of the tube to create pressure, which will aid the removal of ECM.
26. Centrifuge organoids at 200g for 3 min at room temperature.
27. Aspirate the supernatant, and split organoids using either mechanical disruption or Organoid Dissociation Solution (E238001). For mechanical disruption, resuspend the pellet in 1 mL of Organoid Basal Medium. Use a pipette tip to

pipette the organoid suspension up and down 30 times. Use the bottom of the tube to create pressure, which will aid organoid disruption. In case of 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 ≥10 times every 1 min to aid in the disruption of the organoids. Monitor digestion closely to keep the incubation time in Organoid Dissociation Solution to a minimum.

CRITICAL Do not dissociate in Organoid Dissociation Solution for >3 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.

28. After shearing is complete, wash once by topping up with 1 mL of Organoid Basal Medium, and centrifuge at 200g for 3 min at room temperature.
29. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) ECM, and plate organoids in droplets on the bottom of a culture plate as described in Steps 12. After plating is complete, transfer the plate into a humidified incubator at 37 °C and 5% (vol/vol) CO_2 for 15–25 min.
30. Pre-warm Human Intestinal Organoid Maintenance Medium at 37 °C.
31. After the ECM droplets have solidified (15–25 min), carefully pipette pre-warmed medium into the wells.
32. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO2 until the organoids are needed for further experiments.

Appendix 1. Examples of different generations of human intestinal organoids.

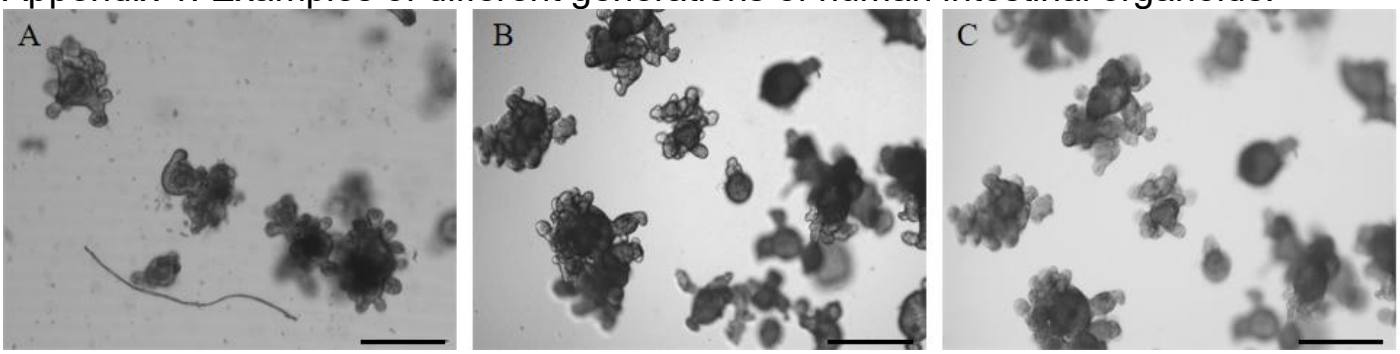

Figure 1. Examples of successful culture in primary, passage and resuscitation of human intestinal organoids.
(A)The growth status of human intestinal organoids in primary culture (P0). The organoids are irregular folds with smooth edges and with obvious budding, and the diameter is about 80-100μm. (B) The growth status of human intestinal organoids in the first passage culture (P1), passage organoids mainly presented vesicle and with obvious budding. (C) The resuscitated cultured organoids showed a steady growth trend and with obvious budding. (scale bar: 200 μm).

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