

bioGenous™ Human Liver Ductal Organoid Kit (Expansion)

Catalog: K2008-HLD

Product Description:

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

Product Information:

Component	Component Cat#	Volume	Storage & Stability
bioGenous™ Human Liver Ductal Organoid Basal Medium (expansion)	K2008-HLD –A100/A500	100mL/500mL	4°C, 12 months
bioGenous™ Human Liver Ductal Organoid Supplement B(50x) (expansion)	K2008-HLD –B100/B500	2mL/10mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Liver Ductal Organoid Supplement C(250x) (expansion)	K2008-HLD –C100/C500	0.4mL/2mL	-20°C, avoid repeated freeze-thaw cycles, 12 months

Materials & Reagents Required But Not Included:

Vender	Materials	Catalog#
bioGenous™	Primary Tissue Storage Solution	K601005
bioGenous™	Epithelial Organoid Basal Medium	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Tissue Digestion Solution	K601008
bioGenous™	Anti-Adherence Rinsing Kit	E238002
bioGenous™	Organoid Cryopreservation Medium (Serum Free)	E238023
Corning®	Matrigel® Growth Factor Reduced Basement Membrane Matrix	356231
	DPBS (1X), liquid, contains no calcium or magnesium	-
	Fetal Bovine Serum (FBS)	-

Preparation of Human Liver Ductal Organoid Expansion Medium and Maintenance Medium

Use a sterile technique to prepare the human liver ductal organoid expansion medium and maintenance medium. hLDs grown in Human Liver Ductal Organoid Expansion Medium overwhelmingly consisted of cholangiocytes. The following example is for preparing 10 mL Expansion Medium and Maintenance Medium. If preparing other volumes, adjust accordingly.

1. Thaw Human Liver Ductal Organoid Supplement B(50x) (Expansion), Human Liver Ductal Organoid Supplement C(250x) (Expansion) 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 Liver Ductal Organoid Expansion Medium. Add 200 µL Human Liver Ductal Organoid Supplement B(50x) (Expansion), 40 µL Human Liver Ductal Organoid Supplement C(250x) (Expansion) to Human Liver Ductal Organoid Basal Medium (Expansion). Mix thoroughly.
NOTE: If not use immediately, store complete medium at 2-8°C for not more than 2 weeks. bioGenous™ Human Liver Ductal Organoid Supplement B (Expansion) contains fungicide and antibiotics(50x).

Protocol for Establishment of Human Liver Ductal 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 liver 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 liver tissue with Epithelial Organoid Basal Medium(B213151) or DPBS until the supernatant is clear.
 4. Before ducts isolation, thaw Matrigel 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 mm³ 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 with a 10 mL pipette at least ten times.
CRITICAL For the subsequent steps, coat the inner surface of every 10 mL pipette with 10% (vol/vol) FBS medium before use to avoid 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 in 37°C 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 appear during digestion.
 10. Once the duct structure appears, stop digestion by transfer the digestion solution into 50mL tube and add 40 ml Epithelial Organoid Basal Medium with 1% FBS.
 11. Stand the tube for 2 min. Transfer the supernatant into a new tube.
 12. Spin the supernatant at 4°C at 300g for 3 min. Aspirate and discard the supernatant.
 13. Re-suspend the pellet with 10 ml DMEM with 1%FBS, and spin at 4°C at 300g for 3 min.
 14. Repeat last step for 2 times.
 15. Aspirate the supernatant and resuspend the pellet in Matrigel. Matrigel should be kept on ice to prevent it from solidifying; thus, work quickly. The amount of Matrigel depends on the size of the pellet. Approximately 20-100 ducts should be plated in 25 µL of Matrigel.
CRITICAL Do not dilute Matrigel too much (Matrigel should be >70% (Matrigel vol/Total vol)) to ensure formation of solid droplets.
 16. Plate the Matrigel 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 Matrigel, proceed with plating as quickly as possible, as the Matrigel may solidify in the tube or pipette tip. Do not let the Matrigel touch the wall of wells.
 17. Place the culture plate into a humidified incubator at 37 °C and 5% (vol/vol) CO₂ for 15-25 min to let the Matrigel solidify.
 18. Prepare the required amount of bioGenous™ Human Liver Ductal Organoid Expansion Medium.
 19. Once the Matrigel 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 Matrigel droplets, as this might disrupt the droplets.
 20. Place the culture plate in a humidified incubator at 37 °C and 5% (vol/vol) CO₂.
 21. Change the medium every 3 days by carefully aspirating medium from the wells and replacing it with fresh, pre-warmed Organoid Complete Medium.
 22. Closely monitor the organoid formation. Ideally, human liver ductal organoids should be passaged for the first time between 5 and 8 days after initial plating.

Splitting and Passaging of Organoids

23. Pipette up and down to disrupt the Matrigel, and transfer the organoid suspension into a 1.5 mL conical tube.
24. 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 Matrigel.
25. Centrifuge organoids at 200g for 3 min at room temperature.
26. 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.
27. 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.

28. Aspirate the supernatant and resuspend the organoid pellet in 70% (vol/vol) Matrigel, 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) CO₂ for 15–25 min.
29. Pre-warm Human Liver Ductal Organoid Maintenance Medium at 37 °C.
30. After the Matrigel droplets have solidified (15–25 min), carefully pipette pre-warmed medium into the wells.
31. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO₂ until the organoids are needed for further experiments.

Last updated on 27th February 2022