bioGenous™ Mouse Liver Ductal Organoid Kit(Expansion)(Serum-free)

Catalog: K2006-MLD

Product Description:

bioGenous™ Mouse Liver Ductal Organoid Kit is a serum-free cell culture medium for the of Mouse ductal organoids (mLDs) derived from adult stem cells. Self-renewal of the ductal epithelium is driven by the proliferation of stem cells and their progenitors located in the liver. mLDs 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 Mouse liver development and disease, mLDs may also have applications in regenerative biology through ex vivo expansion of the ductal epithelia.

Product Information:

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Mouse Liver Ductal Organoid Basal Medium (Expansion)	K2006-MLD -A100/A500	100mL/500mL	2-8℃, 12 months
bioGenous™ Mouse Liver Ductal Organoid Supplement B(50x) (Expansion)	K2006-MLD –B100/B500	2mL/10mL	-20℃, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Mouse Liver Ductal Organoid Supplement C(250x) (Expansion)	K2006-MLD –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™	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
bioGenous™	Organoid Culture ECM (Reduced Growth Factor)	M315066
	DPBS (1X), liquid, contains no calcium or magnesium	-
	Fetal Bovine Serum (FBS)	-

Preparation of Mouse Liver Ductal Organoid Expansion Medium and Maintenance Medium

Use a sterile technique to prepare the mouse liver ductal organoid expansion medium and maintenance medium. mLDs grown in Mouse Liver Ductal Organoid Expansion Medium overwhelmingly consisted of cholangiocytes. The following examples are for preparing 10 mL of Expansion Medium and Maintenance Medium. If preparing other volumes, adjust accordingly.

1. Thaw Mouse Liver Ductal Organoid Supplement B (50x) (Expansion), Mouse 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 Mouse Liver Ductal Organoid Expansion Medium. Add 200 µL Mouse Liver Ductal Organoid Supplement B(50x) (Expansion), 40 µL Mouse Liver Ductal Organoid Supplement C (250x) (Expansion) to 10 mL Mouse Liver Ductal Organoid Basal Medium (Expansion). Mix thoroughly.
   NOTE: If not used immediately, store the complete medium at 2-8°C for not more than 2 weeks. bioGenous™ Mouse Liver Ductal Organoid Supplement B (Expansion) contains fungicides and antibiotics (50x).

Protocol for Establishment of Mouse Liver Ductal Organoids

Establishment of Organoids from Primary Tissue

1. Collect primary mouse 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 is 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 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 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.
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™ Mouse Liver Ductal 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 Mouse Liver Ductal Organoid Medium.
23. Closely monitor organoid formation. Ideally, mouse Liver Ductal organoids should be passaged for the first time between 5 and 8 days after initial plating.

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 Mouse Liver Ductal Organoid Maintenance Medium at 37 °C.
31. After the ECM droplets have solidified (15–25 min), carefully pipette the pre-warmed medium into the wells.
32. Place culture plates in a humidified incubator at 37 °C and 5% (vol/vol) CO^2 until the organoids are needed for further experiments.

Last updated on 20th March 2023