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

Catalog: K2006-MLH

Product Description

bioGenous™ Mouse Liver Ductal (Differentiation) Organoid Kit is a serum-free culture medium for mouse liver ductal (mLDs) organoids derived from adult stem cells. This kit is supposed to be used with bioGenous™ Mouse Liver Ductal (Expansion) Organoid Kit (Serum-free) (K2006-MLD). When cultured in the expansion medium, In mouse liver ductal organoids expansion medium, mLDs organoids predominantly consist of cholangiocytes, characterized by the expression of markers such as Sox9 and Ck19. When the expansion medium is replaced with differentiation medium, mLDs organoids can differentiate into hepatocyte cells, which exhibit hepatocyte-specific markers including Albumin, Cyp3a11, and Mup20. Mouse liver ductal organoids display hallmarks of the ductal epithelium in terms of architecture, cell type composition, and self-renewal dynamics, therefore hold great promise for studies of mouse liver development and disease.

Product Information

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Mouse Liver Ductal (Differentiation) Organoid Basal Medium A	K2006-MLH - A100/A500	100 mL/500 mL	2-8°C,12 months
bioGenous™ Mouse Liver Ductal (Differentiation) Organoid Supplement B (50x)	K2006-MLH – B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Mouse Liver Ductal (Differentiation) Organoid Supplement C (250x)	K2006-MLH – C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Mouse Liver Ductal (Differentiation) Organoid Supplement D (1000x)	K2006-MLH – D100/D500	0.05 mL/0.25 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™	Epithelial Organoid Basal Medium (Serum-free)	B213151
bioGenous™	bioGenousTM Mouse Liver Ductal (Expansion) Organoid Kit	K2006-MLD
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
	DPBS (1x), liquid, contains no calcium or magnesium	-
	Fetal Bovine Serum (FBS)	-

Safety Precautions

Always follow standard laboratory safety procedures when handling biological materials. Wear appropriate personal protective equipment (PPE), including gloves, lab coat, and eye protection. Dispose of waste materials according to local regulations.
For research use only, not for use in diagnostic procedures.

Preparation Before Use

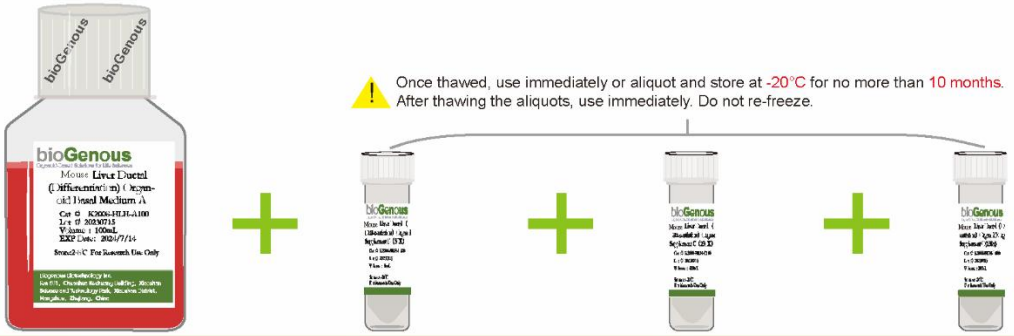
Before initiating the protocol, ensure that all components and equipment are properly prepared:

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 Mouse Liver Ductal Organoid Differentiation Medium

Use the sterile technique to prepare the mouse liver ductal organoid differentiation medium. The following examples are for preparing 10 mL of differentiation medium I and differentiation medium II. If preparing other volumes, adjust accordingly.

Mouse Liver Ductal Organoid Differentiation Medium



Once thawed, use immediately or aliquot and store at -20°C for no more than 10 months.
After thawing the aliquots, use immediately. Do not re-freeze.

I	A: 9.76 mL Mouse Liver Ductal (Differentiation) Organoid Basal Medium	B: 200 µL Mouse Liver Ductal (Differentiation) Organoid Supplement (50X)	C: 40 µL Mouse Liver Ductal (Differentiation) Organoid Supplement (250X)	
II	A: 9.75 mL Mouse Liver Ductal (Differentiation) Organoid Basal Medium	B: 200 µL Mouse Liver Ductal (Differentiation) Organoid Supplement (50X)	C: 40 µL Mouse Liver Ductal (Differentiation) Organoid Supplement (250X)	D: 10 µL Mouse Liver Ductal (Differentiation) Organoid Supplement (1000X)

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

Mouse Liver Ductal Organoid Differentiation Medium:

1. Thaw Mouse Liver Ductal (Differentiation) Organoid Supplement B (50x), Mouse Liver Ductal (Differentiation) Organoid Supplement C (250x) and Mouse Liver Ductal (Differentiation) Organoid Supplement D (250x) on ice. Mix thoroughly.

2. For Mouse Liver Ductal Organoid Differentiation Medium I: Add 200 µL Mouse Liver Ductal (Differentiation) Organoid Supplement B (50x) and 40 µL Mouse Liver Ductal (Differentiation) Organoid Supplement C (250x) to 9.76 mL Mouse Liver Ductal (Differentiation) Organoid Basal Medium. Mix thoroughly.

3. For Mouse Liver Ductal Organoid Differentiation Medium II: Add 200 µL Mouse Liver Ductal (Differentiation) Organoid Supplement B (50x), 40 µL Mouse Liver Ductal (Differentiation) Organoid Supplement C (250x) and 10 µL Mouse Liver Ductal Organoid (Differentiation) Supplement D (250x) to 9.75 mL Mouse Liver Ductal (Differentiation) Organoid Basal Medium. Mix thoroughly.

Protocol for Mouse Liver Ductal Organoids Differentiation

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

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(B213151). 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 mouse liver ductal organoid expansion medium (K2006-MLD) 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.

Mouse Liver Ductal Organoids Differentiation

1. When mouse liver ductal organoids grow to 100-150 μm, changing the culture medium to mouse liver ductal organoids differentiation medium I for 9 days.
2. Change the medium to mouse liver ductal organoid differentiation medium II, and culture for 3 days. During this period, replace the medium every 3 days.
   Note:*If the expression of hepatocyte-specific markers is not significant enough, the cultivation time of medium II can be appropriately extended.*

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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