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

Catalog: K2008-HLH

Product Description

bioGenous™ Human Liver Ductal (Differentiation) Organoid Kit is a serum-free culture medium for differentiation culture of human liver ductal organoids (hLDs) derived from adult stem cells. When cultured in the expansion medium, hLDs predominantly consist of cholangiocytes. Upon switching to a differentiation medium, hLDs can differentiate into hepatocyte cells, expressing key hepatocyte markers such as ALBUMIN, TTR, and CYP3A4. The hLDs faithfully recapitulate the architecture, cell type composition, and self-renewal dynamics of liver ductal epithelium, making them valuable for studying human liver development and disease.

Product Information

Component	Cat#	Volume	Storage& Stability
bioGenous™ Human Liver Ductal (Differentiation) Organoid Basal Medium	K2008-HLH –A100/A500	100 mL/500 mL	4°C, 12 months
bioGenous™ Human Liver Ductal (Differentiation) Organoid Supplement B (50x)	K2008-HLH –B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Liver Ductal (Differentiation) Organoid Supplement C (250x)	K2008-HLH–C100/ C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Liver Ductal (Differentiation) Organoid Supplement D (250x)	K2008-HLH–D100/ D500	0.2 mL/1 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™	Primary Tissue Storage Solution (Serum-free)	K601005
bioGenous™	Epithelial Organoid Basal Medium (Serum-free)	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Tissue Digestion Solution	K601008
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

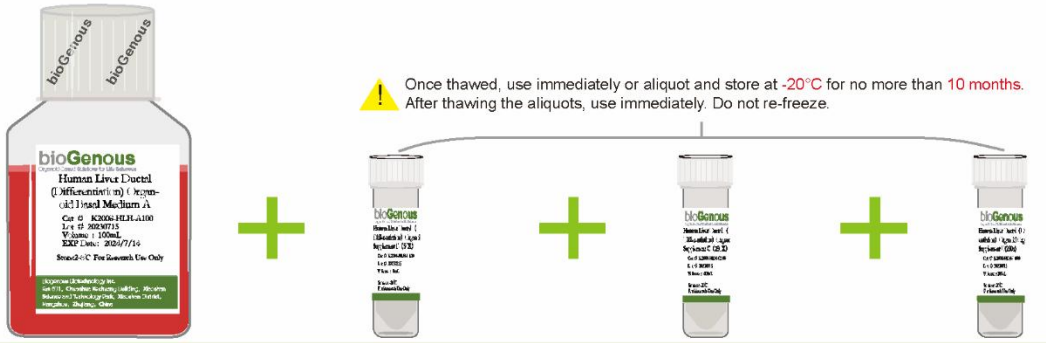
Before initiating the protocol, ensure that all components and equipment are properly prepared:

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 Human Liver Ductal Organoid Differentiation Medium

Use sterile technique to prepare the human 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.

Human 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 Human Liver Ductal (Differentiation) Organoid Basal Medium	B: 200 µL Human Liver Ductal (Differentiation) Organoid Supplement (50X)	C: 40 µL Human Liver Ductal (Differentiation) Organoid Supplement (250X)	
II	A: 9.72 mL Human Liver Ductal (Differentiation) Organoid Basal Medium	B: 200 µL Human Liver Ductal (Differentiation) Organoid Supplement (50X)	C: 40 µL Human Liver Ductal (Differentiation) Organoid Supplement (250X)	D: 40 µL Human Liver Ductal (Differentiation) Organoid Supplement (250X)

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

Human Liver Ductal Organoid Differentiation Medium:

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

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

3. For Human Liver Ductal Organoid Differentiation Medium II: Add 200 µL Human Liver Ductal (Differentiation) Organoid Supplement B (50x), 40 µL Human Liver Ductal (Differentiation) Organoid Supplement C (250x) and 40 µL Human Liver Ductal Organoid (Differentiation) Supplement D (250x) to 9.72 mL Human Liver Ductal (Differentiation) Organoid Basal Medium. Mix thoroughly.

Protocol for Human Liver Ductal Organoids Differentiation

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.

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. 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 human liver ductal organoid differentiation medium I 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.

Human Liver Ductal Organoids Differentiation

1. Culture the human liver ductal organoids in differentiation medium I after seeding for 5 days.
2. Change the medium to human liver ductal organoid differentiation medium II, and culture for 10 days. During this period, replace the medium every 3 days.
3. At the end of this period, the differentiation process is completed.

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.

Last updated on 30th August, 2024