bioGenous™ Human Hepatocyte Organoid Differentiation Kit (Serum-free)

Catalog: K2514-HHD

Product Description

bioGenous™ Human Hepatocyte Organoid Differentiation Kit is a chemically defined, serum-free system engineered to drive the functional maturation of adult hepatocyte-derived organoids. This kit is intended to be used in combination with the bioGenous™ Human Hepatocyte Organoid Expansion Kit (Cat. K2513-HHE). This kit effectively recapitulates the complex metabolic heterogeneity of the liver lobule in vivo. By inducing robust spatial zonation, the kit promotes the differential expression of key metabolic markers, specifically localizing central vein (CV)-like regions (CYP2E1+) and periportal (PV)-like zones (GLUT1+). This high-fidelity model yields mature organoids with superior metabolic activity, providing a highly reproducible and standardized tool for drug metabolism studies, liver disease modeling, and advanced translational research.

Product Information

Component	Cat#	Volume	Storage& Stability
bioGenous™ Human Hepatocyte Organoid Differentiation Basal Medium A	K2514-HHD-A100/A500	100 mL/500 mL	2-8°C, 12 months
bioGenous™ Human Hepatocyte Organoid Differentiation Supplement B (50x)	K2514-HHD-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Hepatocyte Organoid Differentiation Supplement C (250x)	K2514-HHD-C100/C500	0.4 mL/2 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Hepatocyte Organoid Differentiation Supplement D (250x)	K2514-HHD-D100/D500	0.4 mL/2 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™	Human Hepatocyte Organoid Expansion Kit (Serum-free)	K2513-HHE
bioGenous™	Epithelial Organoid Basal Medium (Serum-free)	B213151
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
	Fetal Bovine Serum (FBS)	-
	DPBS (1x), liquid, contains no calcium or magnesium	-

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:

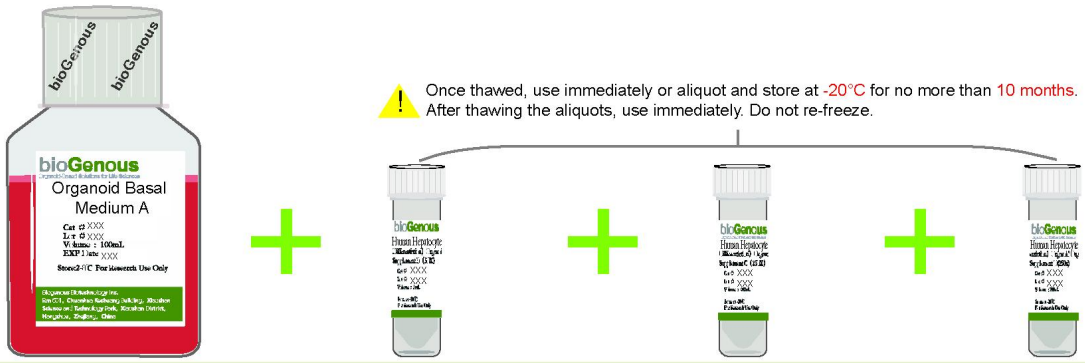
1. Verify that all components are stored according to the guidelines provided in the manual. Avoid repeated freeze-thaw cycles for sensitive reagents. Thaw all necessary reagents according to the instructions. Keep on ice or at the recommended temperature until ready to use.
2. Ensure that all equipment, such as incubators, pipettes, and centrifuges, are calibrated and functioning correctly.

Preparation of Human Hepatocyte Organoid Differentiation Medium

Use sterile technique to prepare the human hepatocyte organoid complete medium. The following examples are for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.

***NOTE**: Two differentiation formulations induce distinct zonation phenotypes. Formulation I drives hepatocyte organoids toward a portal vein-like phenotype, characterized by the expression of PV markers such as HAL and ASS1. Formulation II, supplemented with Component D, activates Wnt signaling and promotes a central vein-like phenotype, with robust expression of CYP2E1 and GLUL. Researchers may choose the appropriate formulation depending on whether periportal or pericentral hepatic functions are desired.*

Human Hepatocyte Organoid Differentiation Medium



I	A: 9.76 mL Human Hepatocyte Organoid Differentiation Basal Medium	B: 200 µL Human Hepatocyte Organoid Differentiation Supplement (50x)	C: 40 µL Human Hepatocyte Organoid Differentiation Supplement (250x)	
II	A: 9.72 mL Human Hepatocyte Organoid Differentiation Basal Medium	B: 200 µL Human Hepatocyte Organoid Differentiation Supplement (50x)	C: 40 µL Human Hepatocyte Organoid Differentiation Supplement (250x)	D: 40 µL Human Hepatocyte Organoid Differentiation Supplement (250x)

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

Human Hepatocyte Organoid Differentiation Medium:

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

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

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

Protocol for Establishment of Differentiated Human Hepatocyte Organoids (dHHO)

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. For differentiation, HHOs must first be expanded for 1.5-2 weeks before switching to the differentiation medium. The following section outlines the pre-expansion process prior to initiating differentiation.
2. Pipette up and down to disrupt the ECM and transfer the successfully established HHOs suspension to a 1.5 mL tube. Continue pipetting up and down to create pressure to help remove the ECM.
3. Centrifuge the tube at 250 x *g* for 3 min at room temperature.
4. 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, incubate at 37°C for 1–3 minutes, pipetting with a filter tip ≥8 times to aid dissociation. 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 >5 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.*
5. 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.
6. 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 10-15 min.
7. Pre-warm the human hepatocyte organoids expansion medium at room temperature.
8. After the ECM droplets have solidified, carefully pipette the pre-warmed medium into the wells.
9. Place the plates in a humidified incubator at 37°C and 5% (vol/vol) CO_2 and culture for 1.5–2 weeks.

Human Hepatocyte Organoids Differentiation

1. Change the medium to human hepatocyte organoid differentiation medium, and culture for 1.5–2 weeks. During this period, replace the medium every 3 days.
2. At the end of this period, the differentiation process is completed. Differentiated hepatocyte organoids (dHHOs) should exhibit mature hepatic features, including expression of CYP3A4, CYP1A2 and CYP2E1, and functional indicators such as bile canaliculi formation and hepatocyte polarity.

Applications

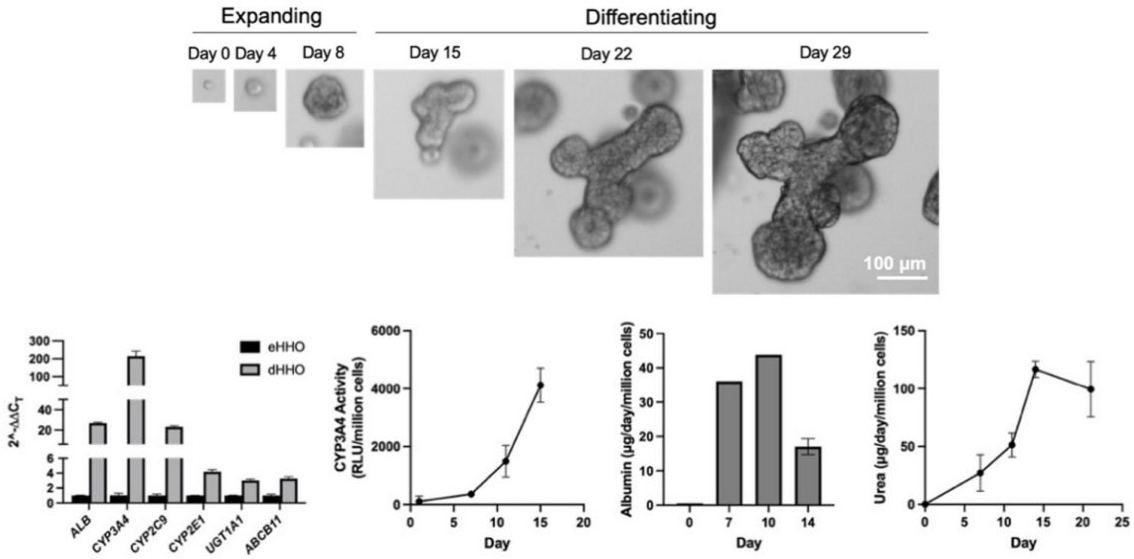


Figure1. The images show human hepatocyte organoids in human hepatocyte organoid differentiation medium from day 15 to day 29 (scale bar: 100 μm). The mRNA expression levels of ALB, CYP3A4, CYP2C9, CYP2E1, and ABCB11 in eHHOs and dHHOs are shown. Cytochrome P450 CYP3A4 enzymatic activity was measured by a chemiluminescence assay at days 0, 5, 10, 15, and 20. Albumin secretion was quantified by ELISA at days 0, 7, 10, and 14 of hepatocyte organoids. Urea secretion was assessed using a colorimetric assay at days 0, 5, 10, 15, and 20. (eHHO: expanding hepatocyte organoids; dHHO: differentiated hepatocyte organoids).

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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