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

Catalog: K2513-HHE

Product Description

bioGenous™ Human Hepatocyte Organoid Expansion Kit is a chemically defined, serum-free medium formulated for the rapid and high-efficiency proliferation of adult liver-derived hepatocyte organoids. This system enables robust, long-term expansion while faithfully preserving the core physiological characteristics of primary hepatocytes, including the sustained expression of canonical markers such as Albumin and HNF4α. By providing a stable and reproducible environment, the kit facilitates the generation of large-scale organoid cultures suitable for high-throughput drug screening, disease modeling, and regenerative medicine research. As a reliable in vitro platform, it serves as an essential foundation for investigating liver development and hepatobiliary pathophysiology within a precisely controlled, serum-free workflow.

Product Information

Component	Cat#	Volume	Storage& Stability
bioGenous™ Human Hepatocyte Organoid Expansion Basal Medium A	K2513-HHE-A100 /A500	100 mL/500 mL	2-8°C, 12 months
bioGenous™ Human Hepatocyte Organoid Expansion Supplement B (50X)	K2513-HHE-B100 /B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Hepatocyte Organoid Expansion Supplement C (250X)	K2513-HHE-C100 /C500	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 Differentiation Kit (Serum-free)	K2514-HHD
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.

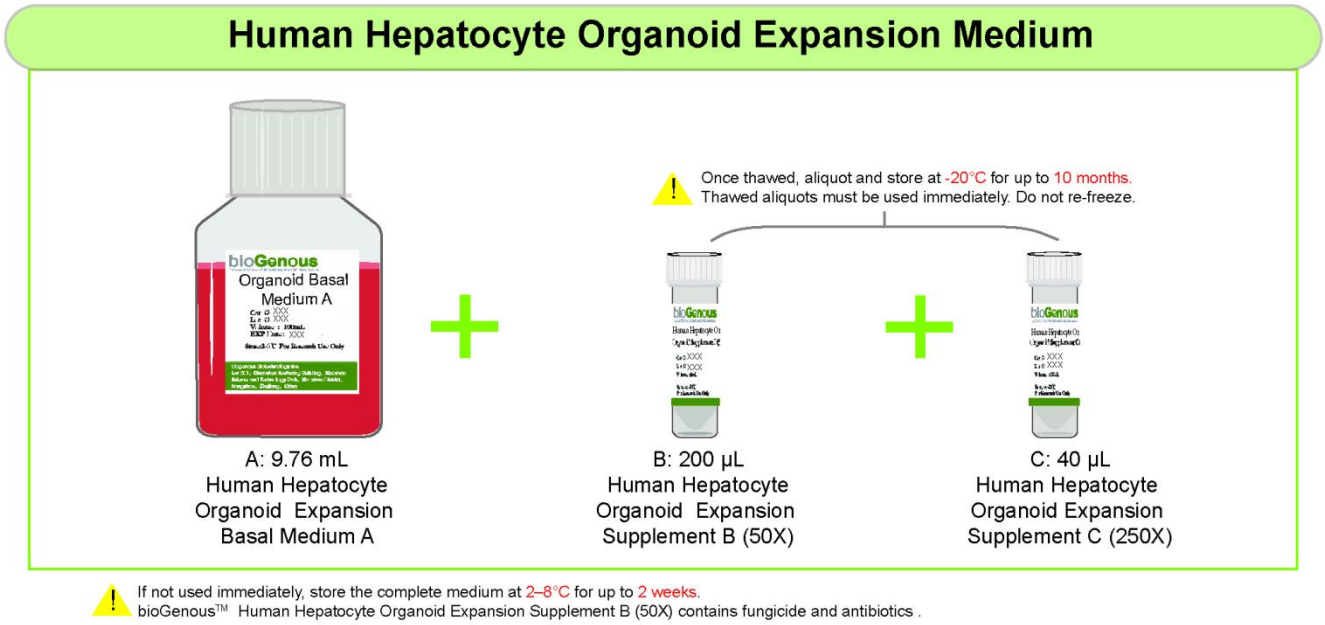
Preparation Before Use

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 Expansion 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.



Human Hepatocyte Organoid Expansion Medium:

1. Thaw Human Hepatocyte Organoid Expansion Supplement B (50X) and Human Hepatocyte Organoid Expansion Supplement C (250X) on ice. Mix thoroughly.

2. Add 200 µL Human Hepatocyte Organoid Expansion Supplement B (50X) and 40 µLHuman Hepatocyte Organoid Expansion Supplement C (250X) to 9.76 mL Human Hepatocyte Organoid Expansion Basal Medium. Mix thoroughly.

Protocol for Establishment of Human Hepatocyte Organoids

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 Human Hepatocytes (PHHs)

1. Obtain primary human hepatocytes (PHHs) either from commercial sources or via freshly isolated and sorted primary hepatocytes. Upon receipt or isolation, keep the PHHs on ice and proceed immediately to culture.
2. Thaw bioGenous™ Organoid Culture ECM (M315066) on ice and keep it cold throughout handling.
3. Resuspend PHHs in cold ECM. Recommended seeding densities are: 1,500 cells in 15 µL ECM per well for 48-well plates or 3,000 cells in 30 µL ECM per well for 24-well plates. ECM should be kept on ice to prevent it from solidifying; thus, work quickly.
   CRITICAL: *Do not dilute ECM below 70% (v/v), as this may compromise droplet stability and organoid formation.*
4. Carefully dispense the cell-ECM mixture as central droplets at the bottom of the wells, avoiding contact with the sidewalls.
   CRITICAL: *Complete this step rapidly to prevent ECM solidification in pipette tips. Do not introduce bubbles.*

5. Incubate the culture plate in a humidified 37°C, 5% CO_2 incubator for 10-15 minutes to allow the ECM to solidify fully.
6. Prewarm the human hepatocyte organoid expansion medium at room temperature.
7. After ECM solidification, gently add 500 µL medium per well by pipetting along the well wall to avoid disturbing the ECM dome.
   CRITICAL: *Do not add medium directly onto the ECM droplet.*
8. Place the plate in a humidified incubator at 37°C, 5% CO_2. Change medium every 3 days, carefully aspirate and replace the medium without touching or disrupting the ECM.
9. Monitor organoid development closely. Hepatocyte organoids should begin forming within the first week.
10. Recommended primary culture duration: about 3 weeks before first passaging.

Splitting and Passaging of Organoids

1. Once organoids have been successfully established and cultured for approximately 3 weeks, they should be passaged every 2–3 weeks depending on growth density and morphology.
2. 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.
3. Centrifuge the tube at 250 x *g* for 3 min at room temperature.
4. Aspirate the supernatant and split the organoids using either bioGenous™ 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 min, 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 bioGenous™ 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 >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 culture plates in a humidified incubator at 37°C and 5% (vol/vol) CO_2 until the organoids are needed for further experiments.
10. During passaging, the appearance of vacuolated ductal organoids may be observed. These can be selectively removed by clonal picking to enrich and maintain purified hepatocyte organoids.

Applications

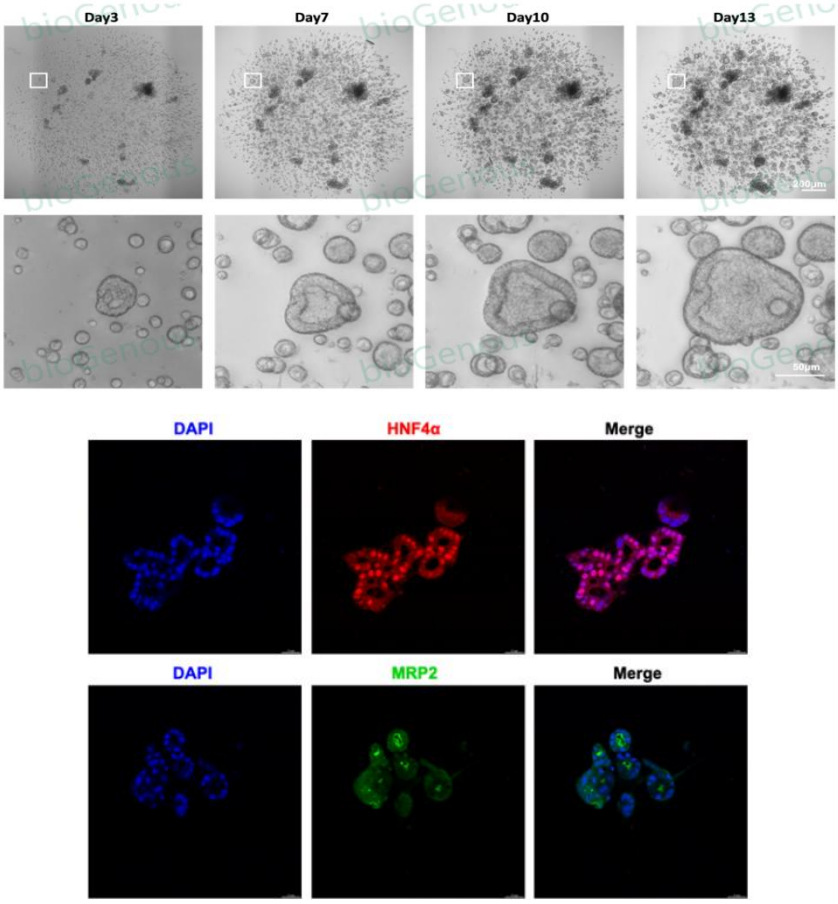


Figure 1.The images show hepatocyte organoids cultured in human hepatocyte expansion medium at Day 3, 7, 10, and 13. Hepatocyte organoids immunostained with HNF4α (Red) and MRP2 (Green).

Quality Control

All components are negative for bacterial and fungal contamination. Certificates 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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