

# bioGenous<sup>™</sup> Human Brain (Expansion) Organoid Kit (Serum-free) Catalog: K-2603

#### **Product Description**

bioGenous<sup>TM</sup> Human Brain (Expansion) Organoid Kit is a serum-free culture medium for human brain organoids (hFBs). In the human brain organoid expansion medium, human brain tissue self-organizes into hFBs under in vitro conditions, successfully replicating key features of in vivo cellular heterogeneity and intricate tissue architecture. These hFBs demonstrate capacity for prolonged expansion, a process fundamentally dependent on preserved tissue integrity. This structural preservation facilitates the generation of a native-like extracellular matrix microenvironment that critically enables sustained hFBs proliferation. hFBs may also have applications in regenerative biology and help understand developmental and disease-related biology.

#### **Product Information**

Component	Catalog#	Volume	Storage& Stability
bioGenous™ Human Brain			
(Expansion) Organoid Basal	K-2603-A100/A500	100 mL/500 mL	2-8°C, 12 months
Medium			
bioGenous™ Human Brain			-20°C, avoid repeated freeze-
(Expansion) Organoid	K-2603-B100/B500	4 mL/20 mL	thaw cycles, 12 months
Supplement B (25x)			thaw cycles, 12 months
bioGenous™ Human Brain			-20°C, avoid repeated freeze-
(Expansion) Organoid	K-2603-C100/C500	0.4 mL/2 mL	thaw cycles, 12 months
Supplement C (250x)			ulaw cycles, 12 monus

### 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™	Anti-Adherence Rinsing Solution	E238002
	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

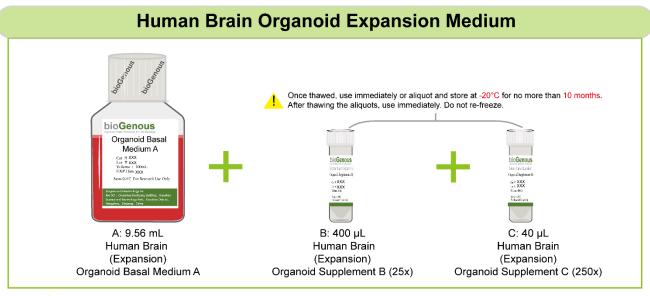
Before initiating the protocol, ensure that all components and equipment are properly prepared:

- 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.
- Ensure that all equipment, such as incubators, pipettes, and centrifuges, are calibrated and functioning correctly.



# Preparation of Human Brain Organoid Expansion Medium

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



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If not use immediately, store complete medium at 2-8°C for no more than 2 weeks. bioGenous™ Human Brain (Expansion) Organoid Supplement B (25x) contains fungicide and antibiotics

#### Human Brain (Expansion) Organoid Medium:

- 1. Thaw Human Brain (Expansion) Organoid Supplement B (25x) and Human Brain (Expansion) Organoid Supplement C (250x) on ice. Mix thoroughly.
- 2. Add 400  $\mu$ L Human Brain (Expansion) Organoid Supplement B (25x) and 40  $\mu$ L Human Brain (Expansion) Organoid Supplement C (250x) to 9.56 mL Human Brain (Expansion) Organoid Basal Medium. Mix thoroughly.

# Protocol for Establishment of Human Brain Organoids



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

#### **Establishment of Organoids from Primary Tissue**

- Collect primary human brain tissue in ice-cold Primary Tissue Storage Solution (K601005) using conical tubes.
   Keep tissue samples at 4°C until the start of the isolation.
- Assess whether the obtained tissue consist purely of cerebral cortex. Remove non-epithelial components as much as possible using surgical scissors or scalpels and forceps under a dissection microscope. Otherwise, continue to the Step 3.
- 3. Rinse the tissue with Epithelial Organoid Basal Medium (B213151).
- 4. Mince the tissue into small fragments of 0.8-1 mm in a cell culture dish using surgical scissors or scalpels.
- 5. Allow to settle in the cell culture dish for 1-2 minutes, aspirate the supernatant and resuspend the fragments in Epithelial Organoid Basal Medium.
- Repeat Step 5 once more time.
- 7. Aspirate the supernatant, distribute tissue fragments into 6-well plates which pre-treated with Anti-Adherence Rinsing Solution (E238002) around 20-30 fragments/well.
  - **CRITICAL:** Trim 3-5 mm from the tip of a pipette tip using sterile scissors before aspirating fragments. Maintain vertical cutting angle to ensure smooth fragment flow.
- 8. Prepare the required amount of human brain organoid expansion medium.
- 9. Place the culture plate under constant rotation (90 rpm) on orbital shaker in a humidified incubator at 37°C and 5% (vol/vol) CO<sub>2</sub>.



- 10. Change the medium every 2-3 days by carefully aspirating the medium from the wells and replacing it with a fresh, pre-warmed organoid expansion medium.
- 11. Closely monitor the organoid formation. Ideally, human brain organoids should be passaged until they reached a size of approximately 2-3 mm in diameter. Typical morphologies of successfully cultured human brain organoids are shown in Figure 1.

#### **Splitting and Passaging of Organoids**

- Pre-warm the human brain organoid expansion medium at 37°C.
- 2. Aspirate the supernatant and split the organoids by mechanical disruption.
  Mechanical disruption-based organoids dissociation: Resuspend the organoids in 1.5 mL of Epithelial Organoid Basal Medium. Carefully mince the organoids into small fragments of 0.8-1 mm in a cell culture dish.
  CRITICAL: Trim 3-5 mm from the tip of a pipette tip using sterile scissors before aspirating fragments.
  Maintain vertical cutting angle to ensure smooth fragment flow.
- 3. Aspirate the supernatant, distribute organoids fragments into 6-well plates which pre-treated with Anti-Adherence Rinsing Solution (E238002) and carefully pipette the pre-warmed medium into the wells.
- 4. Place the culture plate under constant rotation (90 rpm) on orbital shaker in a humidified incubator at 37°C and 5% (vol/vol) CO<sub>2</sub>.

### **Applications**

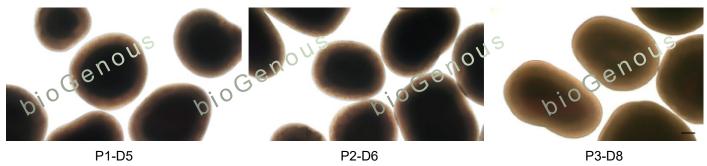


Figure 1. Images of human brain organoids across different passages. Passage number, as well as days post culture in the passage, are indicated below each image. Scale bar: 200 µm.

# **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.

#### Disclaimer

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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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