

Human Glioma Cancer Organoid Growth Medium

Introduction

Patient-derived tumor organoids can retain the genetic characteristics of the primary tumor, which have a wide range of applications in disease modeling, drug screening, and exploration of tumor development mechanisms. This medium is a chemically defined, serum-free culture medium for the establishment and maintenance of human glioma cancer organoids. Additionally, this product is a ready-to-use medium requiring no additional factors, which is more convenient for use.

Components and Storage

Components	Size	100 mL	500 mL	Storage
Human Glioma Cancer Organoid Growth Medium		100 mL	500 mL	-20°C
Shipping: Dry ice		Shelf life: 6 months		

Materials Required but Not Included

Products	Catalog number
Organoid Wash Buffer	K2846
Tissue Dissociation Solution	K2841
GFR Basement Membrane Matrix (Phenol Red-Free)	EM1001
Organoid Dissociation Solution	K2843
Organoid Cryopreservation Medium	K2844
100 µm cell strainer	-
24-well cell culture plate	-
15, 50 mL centrifuge tubes	-
1.5 mL EP tubes	-

Protocol

- Preparation before use:** Thaw components stored at -20°C in advance, and aliquot the Human Glioma Cancer Organoid Growth Medium to avoid repeated freeze-thaw cycles.
- Construction of organoids from primary tissues**

1. Tissue isolation

- 1) Isolate tissue in accordance with local ethics regulations. Under sterile conditions, isolate tumor tissue, wash tissue with saline several times, and then place in a conical tube containing Tissue Storage Solution. Keep tissue at 4°C until start the experiment.

***Note:** For detailed protocol, please refer to the manual of Tissue Storage Solution (K2842).

- 2) Cut the tissue into the size of a grain of rice (2-4 mm³) with surgical scissors.
- 3) Add 5-10 mL of Organoid Wash Buffer to repeatedly pipette and wash.
- 4) Remove the Organoid Wash Buffer.
- 5) Repeat steps 3-4 for a total of 5-10 wash of tissue.

2. Raw tissue analysis (optional)

- 1) If additional immunoassays are required, take 1-2 tissue blocks and fix with a fixative solution (4% PFA or 10% formalin).
- 2) For molecular analysis (whole exome/genome/mRNA sequencing) or biochemical analysis (WB or proteomics), soak 1-2 tissue blocks in RNAlater™ and freeze them at -80°C.

3. Tissue cutting

- 1) Transfer tissue to a 1.5 mL EP tube and further cut into 1mm pieces with surgical scissors.
- 2) Transfer pieces to a 15 mL centrifuge tube.
- 3) Add 2 mL of Red Blood Cell Lysis Buffer (K1169) to resuspend the precipitate, and then let it stand at room temperature for 3 min.

***Note:** If the tissue has been harvested for more than 48 h, it is not recommended to use Red Blood Cell Lysis Buffer.

- 4) Add 10 mL of Organoid Wash Buffer. Centrifuge at 200 g for 5 min and discard the supernatant.

4. Organoid culture

- 1) Mix the pellet and room temperature Human Glioma Cancer Organoid Growth Medium on ice at a ratio of 1:20.

***Note:** Equilibrate Human Glioma Cancer Organoid Growth Medium at room temperature for 15 min in advance.

- 2) Incubate the plate in a 37°C, 5% CO₂ orbital shaker. Or add 500 µL of medium per well to Ultra-Low Attachment plates and then incubate in a 37°C, 5% CO₂ incubator.
- 3) Exchange Human Glioma Cancer Organoid Growth Medium every 2-4 days.

***Note:**

- a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.

- b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells and replace with Human Glioma Cancer Organoid Growth Medium after 4 h.

3. Organoid passage

1. Organoid harvest

- 1) Discard the medium and slowly add pre-chilled Organoid Wash Buffer to the wells (PBS containing 1% antibiotics can also be used).
- 2) Pipet Matrigel/Organoid/Medium mixture up and down to release organoid from the Matrigel.

***Note:** If finding that organoids are attached to the wall, scrape the organoid off with a pipette tip.

- 3) Transfer the mixture to a new 15 mL tube.
- 4) Centrifuge at 300 g or 1500 rpm for 5 min, and discard the supernatant.

2. Organoid dissociation

- 1) Suspend pellet in 1-2 mL of Organoid Wash Buffer. Pipet the suspension up and down.
- 2) When observing the organoids reach 40-200 μm in diameter, stop the digestion.
- 3) Add Organoid Wash Buffer to a total volume of 12 mL.
- 4) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

3. Organoid passage

- 1) Mix the pellet and room temperature Human Glioma Cancer Organoid Growth Medium on ice at a ratio of 1:20.

***Note:** Equilibrate Human Glioma Cancer Organoid Growth Medium at room temperature for 15 min in advance.

- 2) Incubate the plate in a 37°C, 5% CO₂ orbital shaker. Or add 500 μL of medium per well to Ultra-Low Attachment plates and then incubate in a 37°C, 5% CO₂ incubator.
- 3) Exchange Human Glioma Cancer Organoid Growth Medium every 2-4 days.

***Note:**

- a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.
- b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells and replace with Human Glioma Cancer Organoid Growth Medium after 4 h.

4. Organoid cryopreservation

Cryopreservation must be performed when the organoids are in optimal condition. Fast-growing organoids (up to 300-500 μm in diameter) can be cryopreserved when they reach 150-200 μm in diameter. Slow-growing organoids (up to 100-300 μm in diameter) can be cryopreserved when they reach 100-150 μm in diameter.

During the cryopreservation, gently pipetting to ensure the integrity of the organoids.

1. Organoid harvest

- 1) Discard the medium and slowly add pre-chilled Organoid Wash Buffer to the wells (PBS containing 1% antibiotics can also be used).
- 1) Pipet Matrigel/Organoid/Medium mixture up and down to release organoid from the Matrigel.

***Note:** If finding that organoids are attached to the wall, scrape the organoid off with a pipette tip.

- 2) Transfer the mixture to a new 15 mL tube.
- 3) Centrifuge at 300 g or 1500 rpm for 5 min, and discard the supernatant.

2. Organoid cryopreservation

- 1) Depending on the pellet volume, mix the pellet and Organoid Cryopreservation Medium at a ratio of 1:10.
- 2) After mixing, aliquot the organoids into cryopreservation vials, and perform cryopreservation by programmed cooling.

***Note:** Cryopreserved organoids can be stored at -80°C for one month, and for long-term storage, store the vials in a liquid nitrogen tank.

3. Organoid resuscitation

- 1) Equilibrate Organoid Wash Buffer to room temperature in advance. Add 5 mL of room temperature Organoid Wash Buffer in a 15 mL centrifuge tube. 2% serum can be added to the Organoid Wash Buffer.
- 2) Remove the vials from the liquid nitrogen tank and thaw them in a 37°C water bath for 1-2 min.
- 3) Slowly transfer the thawed organoid suspension to the 15 mL centrifuge tube containing Organoid Wash Buffer. Add another 5 mL of Organoid Wash Buffer.
- 4) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.
- 5) Add 2 mL of room temperature Organoid Wash Buffer (without serum) and gently resuspend the organoids.
- 6) Add 8 mL of Organoid Wash Buffer.
- 7) Centrifuge at 300 g or 1500 rpm for 5 min. Discard the supernatant.

4. Organoid culture

- 1) Mix the pellet and room temperature Human Glioma Cancer Organoid Growth Medium on ice at a ratio of 1:20.

***Note:** Equilibrate Human Glioma Cancer Organoid Growth Medium at room temperature for 15 min in advance.

- 2) Incubate the plate in a 37°C, 5% CO₂ orbital shaker. Or add 500 µL of medium per well to Ultra-Low Attachment plates and then incubate in a 37°C, 5% CO₂ incubator.
- 3) Exchange Human Glioma Cancer Organoid Growth Medium every 2-4 days.

***Note:**

- a) The time for exchanging medium depends on the experimental situation. If the cell density is high, the medium turns yellow after one day, and it is necessary to change the medium daily and dilute the cell density as soon as possible.
- b) If microbial contamination occurs during the incubation process, add 1 mL of 3.5 mM NaOH solution to the contaminated wells and replace with Human Glioma Cancer Organoid Growth Medium after 4 h.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology

www.apexbt.com

7505 Fannin street, Suite 410, Houston, TX 77054.

Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com