bioGenous™ OrganoidpleX Medium (Serum-free)

Catalog: CO1233

Product Description

bioGenous™ OrganoidpleX Medium (Serum-free) is a well-formulated reduced-serum culture medium intended for the ex vivo coculture and maintenance of organoids and human immune cells, under reduced serum conditions. This medium contains essential nutrients that closely mimic the tumour microenvironment and hence supports the short-term ex vivo maintenance of complex coculture models that involve patient-derived tumour organoids and immune cells such as human peripheral blood mononuclear cells (PBMCs), tumour-infiltrating lymphocytes (TILs), or natural killer cells (NK) during drug screening applications. It is suitable for short-term coculture of immune cells and organoids such as colorectal, gastric, lung cancer, among others.

Product Information

Component	Component Cat#	Volume	Storage & Stability
bioGenous™ OrganoidpleX Basal Medium A	CO1233-A100/A500	100 mL/500 mL	2-8°C，12 months
bioGenous™ OrganoidpleX Supplement B（50X）	CO1233-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ OrganoidpleX Supplement C (250X)	CO1233-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	Reagents	Catalog#
bioGenous™	Organoid Cryopreservation Medium（Serum Free）	E238023
bioGenous™	Cancer Organoid Basal Medium	B213152
bioGenous™	Anti-Adherence Rinsing Solution	E238002
bioGenous™	Organoid Dissociation Solution	E238001
	Fetal Bovine Serum (FBS)	
	DPBS (1X), liquid, contains no calcium or magnesium	
	Ficoll, liquid, density gradient centrifugation medium	

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 media and 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 Endometrial Cancer Organoid Complete Medium

To prepare the OrganoidpleX medium, it is important to work in an aseptic condition. Depending on the experimental requirement, the protocol may be optimized accordingly. The following example outlines the preparation of a 10 mL complete medium.

bioGenous™ OrganoidpleX Medium

A: 9.76 mL
OrganoidpleX
Basal Medium A

B: 200 µL
OrganoidpleX
Supplement B (50x)

C: 40 µL
OrganoidpleX
Supplement C (250x)

⚠ Once thawed, use immediately or aliquot and store at -20 °C for no more than 10months. After thawing the aliquots, use immediately. Do not re-freeze.

⚠ If not used immediately, store the complete OrganoidpleX medium at 2-8°C for no more than 2 weeks. bioGenous™ OrganoidpleX Supplement B (50x) contains fungicide and antibiotics.

bioGenous™ OrganoidpleX Medium：

1. Thaw the OrganoidpleX Supplement B (50x) and OrganoidpleX Supplement C (250x) on ice. Mix thoroughly.

2. Add 200µL OrganoidpleX Supplement B (50x) and 40µL OrganoidpleX Supplement C (250x) to 9.76mL of OrganoidpleX Basal Medium and supplement with 1% FBS. Mix thoroughly.

For coculture models over 24 hours for suspension cultures, it is recommended to supplement the medium with 1% ECM or Matrigel and mix thoroughly before use.

Organoid-Immune Cell Coculture （Using PDO-PBMC as an example）

⚠ 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.

Extraction of Peripheral Blood Mononuclear Cell:

1. Dilute the anticoagulated whole blood sample 1:1 with PBS (the dilution ratio can be adjusted according to specific circumstances).
2. Add an equal volume of Ficoll separation medium to the bottom of the centrifuge tube, minimizing adhesion to the tube wall as much as possible.
3. Slowly add the whole blood sample along the tube wall into the centrifuge tube containing Ficoll, avoiding disturbing the liquid surface to form distinct layers.
4. Centrifuge at 400g for 30 minutes with slow deceleration.
5. Remove the centrifuge tube to obtain distinct layers, from top to bottom: plasma layer, PBMC layer, Ficoll, granulocyte layer, and erythrocyte layer. Carefully remove the PBMC layer and transfer it to a new centrifuge tube.
6. Add 2 volumes of PBS, centrifuge at 500g for 5 minutes, and wash twice.

7. Resuspend in PBS containing 2% serum, perform cell counting, and set aside. The PBMCs extracted in this step include lymphocytes (such as T cells, B cells, natural killer cells) and monocytes, which can be further activated or cultured in immune cell culture medium according to experimental requirements.

Preparations of intended organoids and subsequent co-culture methods:

1. Culture the intended cancer organoids to maturity and ensure that they have gone through at least 2 passages. Organoids meeting above requirements are eligible for co-culture experiment.
2. Pre-cool the basal medium at 4°C in advance, add 1 mL of basal medium to each well of the intended organoids, and gently scrape off the Matrigel.
3. Centrifuge at 300g for 3 minutes, remove the supernatant and excess Matrigel, and repeat the washing twice if necessary. In this way we can achieve dissociated organoids, and the complete organoids are recommended for the later immune cell infiltration experiment.
4. Mix the suspensions of both cell types based on the desired effector-to-target ratio as per experimental requirements and seed into appropriate culture plates or dishes.
5. Incubate in a 37°C, 5% CO2 humidified incubator, and monitor cell morphology daily.
6. Assess immune cytotoxicity through various methods according to experimental need (e.g., ELISA, confocal microscopy, high-content imaging among others).
   NOTE: *bioGenous™ OrganoidpleX is for short-term coculture of tumour organoids and immune cells during drug evaluation. It is not intended for long-term cultures beyond 5 days.*

Related Products

Vender	Materials	Catalog#
bioGenous™	T Cell Expansion Medium	SFM-T001
bioGenous™	NK Cell Expansion Medium	SFM-NK001

References

1. Magré L, Verstegen MMA, Buschow S, van der Laan LJW, Peppelenbosch M, Desai J. Emerging organoid-immune co-culture models for cancer research: from oncoimmunology to personalized immunotherapies. J Immunother Cancer. 2023;11(5): e006290. doi: 10.1136/jitc-2022-006290.
2. Dijkstra KK, Cattaneo CM, Weeber F, Chalabi M, van de Haar J, Fanchi LF, et al. Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. Cell. 2018;174(6): 1586-1598.e12. doi: 10.1016/j.cell.2018.07.009.
3. Cattaneo CM, Dijkstra KK, Fanchi LF, Kelderman S, Kaing S, van Rooij N, van den Brink S, Schumacher TN, Voest EE. Tumor organoid-T-cell coculture systems. Nat Protoc. 2020;15(1): 15-39. doi: 10.1038/s41596-019-0232-9.

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

For research use only, not for use in diagnostic procedures. Read the Safety Data Sheets (SDSs) and follow the manufacture's instruction.

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For questions, suggestions, and technical supports, please contact us at E-mail: info@biogenous.cn.

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