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

Catalog: K2150-HAD

Product Description

bioGenous™ Human Airway (Differentiation) Organoid Kit is a serum-free culture medium designed for the differentiation of human airway organoids (hAOs) derived from human airway stem cells. When cultured in differentiation conditions for 10-14 days, airway organoids develop well-defined luminal structures, within which extensive ciliary beating or accumulation of secretory material can be observed under the microscope. Differentiated hAOs exhibit key functional and structural features of the mature airway epithelium, including high expression of tubulin in motile cilia and MUC5AC in mucus-secreting goblet cells. In combination with **bioGenous™ Human Airway (Expansion) Organoid Kit**, this differentiation system enables robust and reproducible generation of airway epithelium in vitro, supporting a wide range of applications in respiratory research, toxicology, drug discovery, and regenerative medicine.

Product Information

Component	Cat#	Volume	Storage & Stability
bioGenous™ Human Airway (Differentiation) Organoid Basal Medium	K2150-HAD-A100/A500	100 mL/500 mL	2-8°C, 12 months
bioGenous™ Human Airway (Differentiation) Organoid Supplement B (50x)	K2150-HAD-B100/B500	2 mL/10 mL	-20°C, avoid repeated freeze-thaw cycles, 12 months
bioGenous™ Human Airway (Differentiation) Organoid Supplement C (250x)	K2150-HAD-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™	Tissue Digestion Solution (Serum-free)	K601008
bioGenous™	Primary Tissue Storage Solution (Serum-free)	K601005
bioGenous™	Epithelial Organoid Basal Medium	B213151
bioGenous™	Organoid Dissociation Solution	E238001
bioGenous™	Red Blood Cell Lysis Solution	E238010
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	-
	100 μm cell strainer	-

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.

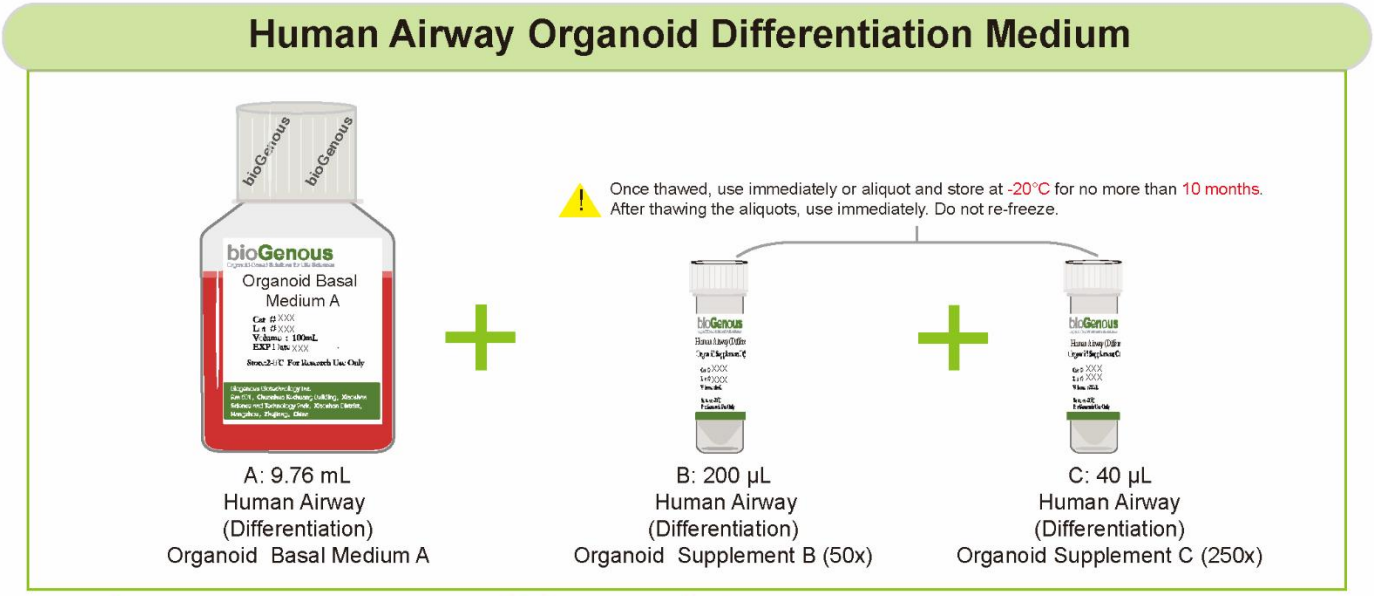
Preparation Before Use

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 Airway Organoid Differentiation Medium

Use a sterile technique to prepare the human airway organoid differentiation medium. The following example is for preparing a 10 mL differentiation medium. If preparing other volumes, adjust accordingly.



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

Human Airway Organoid Differentiation Medium:

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

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

Protocol for Human Airway Organoids Differentiation

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. 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.
2. Centrifuge the tube at 250 x *g* for 3 min at room temperature.
3. 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, pipette up and down and incubate at 37°C until the organoids are released from the ECM. Pipette up and down with a filter tip for ≥8 times every 2 min to aid in the disruption of the organoids. 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 >7 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.*

4. 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.
5. 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 15-25 min.
6. Pre-warm the Human Airway Organoid Complete Medium (bioGenous™ Human Airway Organoid Kit (Serum-free), No. K2018-HA) at 37°C.
7. After the ECM droplets have solidified (15-25 min), carefully pipette the pre-warmed medium into the wells.
8. 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.

Human Airway Organoids Differentiation

1. Culture the human airway organoids in complete medium after seeding for 3 days.
2. Change the medium to human airway organoid differentiation medium, and culture for 10-14 days. During this period, replace the medium every 3 days.
   Note*: If the organoid density is low during the expansion phase, differentiation medium can be directly applied once the organoid diameter reaches 150 μm or larger. If the density is high, organoids can be diluted and replated before switching to differentiation medium.*

3. After 10-14 days of differentiation culture, evident ciliary beating or accumulation of secretory materialwithin the lumens of airway organoids can be observed. At this stage, differentiation can be terminated, and samples can be collected.

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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