PneumaCult™ Airway Organoid Kit

Serum- and BPE-free medium kit for differentiation of human airway epithelial cells into mature airway organoids

Catalog #05060	1 Kit
#05061	450 mL
#05062	10 mL
#05063	40 mL

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Product Description

PneumaCult™ Airway Organoid Kit is a serum- and BPE-free culture medium system for efficient establishment and differentiation of airway organoids derived from human bronchial epithelial cells (HBECs). After 2D expansion in PneumaCult™-Ex Plus Medium, PneumaCult™ Airway Organoid Seeding Medium allows for initiation of 3D organoid culture, then PneumaCult™ Airway Organoid Differentiation Medium is used to obtain morphologically relevant airway organoids. Airway organoids provide a functional in vitro organotypic culture system for studying the airway epithelium. When fully differentiated, the organoids exhibit a centralized lumen surrounded by a polarized airway epithelial cell layer, comprised of differentiated cell types, such as ciliated cells and goblet cells. Applications of airway organoid cultures include modeling lung diseases, drug screening for efficacy and toxicity, and studying the functions of the airway epithelium.

Should you intend to use this product for commercial purposes, please contact HUB at www.huborganoids.nl for a commercial use license or for clarification in relation to HUB licensing.

Product Information

The following products are sold as a complete kit (Catalog #05060) and are also available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
PneumaCult™ Airway Organoid Basal Medium	05061	450 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
PneumaCult™ Airway Organoid Seeding Supplement*	05062	10 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
PneumaCult™ Airway Organoid Differentiation Supplement*	05063	40 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free*	Corning 356231
D-PBS (Without Ca++ and Mg++)	37350
Falcon® 25 cm^2 Rectangular Canted Neck Cell Culture Flask with Vented Cap	Corning 353109
Falcon® Conical Tubes, 15 mL	38009
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
PneumaCult™-Ex Plus Medium	05040
Tissue culture-treated 24-well flat-bottom plate	e.g. 38017
Trypan Blue	07050

*For best results, use Matrigel® with protein concentration > 8.5 mg/mL.

Preparation of Media

PneumaCult™ Airway Organoid Seeding Medium

Use sterile technique to prepare PneumaCult™ Airway Organoid Seeding Medium (Basal Medium + Seeding Supplement). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw PneumaCult™ Airway Organoid Seeding Supplement at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 10 mL PneumaCult™ Airway Organoid Seeding Supplement to 90 mL PneumaCult™ Airway Organoid Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete medium at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the individual components.
3. Immediately before use, add desired antibiotics.

PneumaCult™ Airway Organoid Differentiation Medium (ODM)

Use sterile technique to prepare PneumaCult™ Airway ODM (Basal Medium + Differentiation Supplement + Heparin Solution + Hydrocortisone Stock Solution). The following example is for preparing 400 mL of complete medium. If preparing other volumes, adjust accordingly.

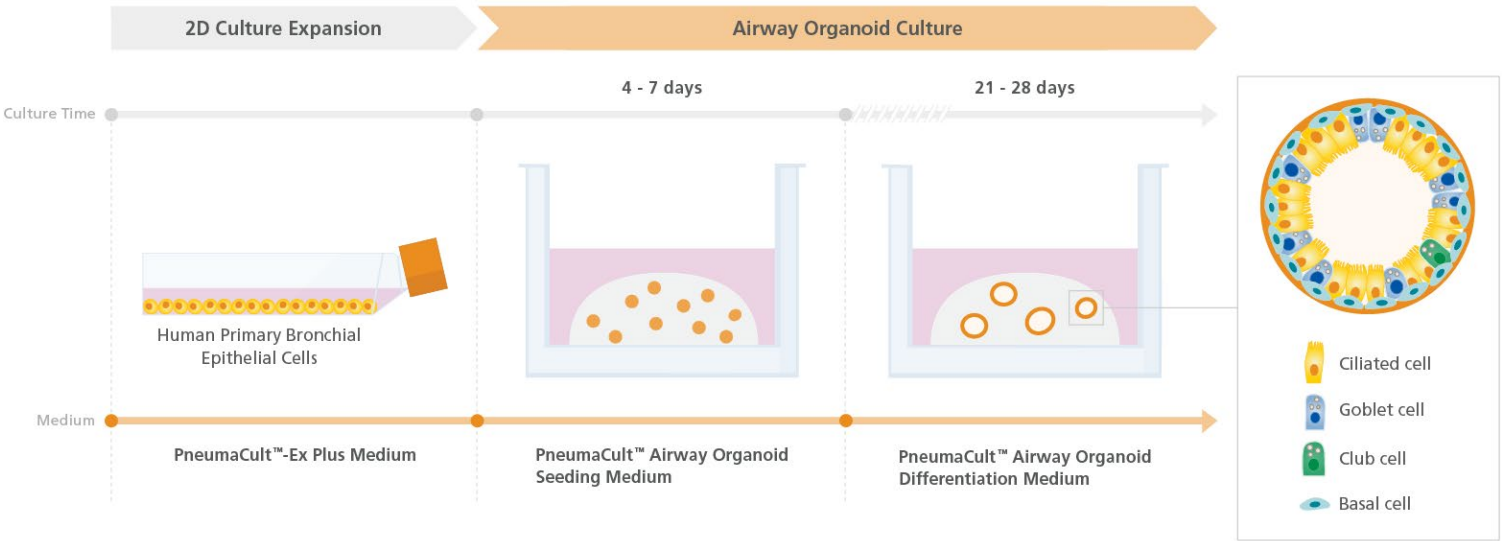
1. Thaw PneumaCult™ Airway Organoid Differentiation Supplement at room temperature (15 - 25°C). Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 40 mL of PneumaCult™ Airway Organoid Differentiation Supplement to 360 mL of PneumaCult™ Airway Organoid Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the individual components.
3. Immediately before use, add the following to the medium prepared in step 2:
   - 800 µL Heparin Solution
   - 2 mL Hydrocortisone Solution
   - Desired antibiotics

   Mix thoroughly.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

A. 2D HBEC Expansion in PneumaCult™-Ex Plus Medium

B. Passaging 2D HBEC Expansion Cultures in PneumaCult™-Ex Plus Medium

C. 3D HBEC Expansion in PneumaCult™ Airway Organoid Seeding Medium

D. 3D HBEC Differentiation in PneumaCult™ Airway ODM

A. 2D HBEC Expansion in PneumaCult™-Ex Plus Medium

For best results, use early-passage HBECs. If starting from cryopreserved cells, thaw cells directly into complete PneumaCult™-Ex Plus Medium.

1. Day 0
   a. Prepare PneumaCult™-Ex Plus Medium as described in the corresponding Product Information Sheet (PIS). Warm to 37°C.
   b. Add 1.25 x 10^5 HBECs to a T-25 cm^2 flask containing 5 mL warm (37°C) PneumaCult™-Ex Plus Medium. Incubate at 37°C and 5% CO_2.

      NOTE: If starting from cryopreserved cells, perform a full-medium change 24 hours after initial plating.
2. Day 2: Perform a full-medium change. Incubate at 37°C and 5% CO_2. Place a 24-well tissue culture-treated plate in the incubator, for use in section C; this plate must warm to 37°C overnight at a minimum.
3. Perform a full-medium change every 2 days until cells are 50 - 60% confluent and ready to be passaged. This typically takes 3 - 5 days.

   NOTE: This expansion phase may take longer for some donor cell populations. On weekends, change the medium on Friday afternoon then first thing on Monday morning.
4. Proceed to section B for passaging.

B. Passaging 2D HBEC Expansion Cultures in PneumaCult™-Ex Plus Medium

When the HBEC expansion culture has reached 50 - 60% confluency, perform the following preparation steps for initiation of 3D HBEC expansion in section C:

- Place a box of sterile 200 µL pipette tips at 2 - 8°C.
- Thaw Matrigel® on ice.

  NOTE: Keep Matrigel® on ice when thawing and handling to prevent it from solidifying.
- Prepare PneumaCult™ Airway Organoid Seeding Medium and warm to room temperature (15 - 25°C).

Passage the HBEC expansion culture as follows:

1. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™-Ex Plus Medium, ACF Enzymatic Dissociation Solution, and ACF Enzyme Inhibition Solution to room temperature.
2. Aspirate medium from the HBEC culture. Add 5 mL of D-PBS (Without Ca++ and Mg++) to wash cells. Aspirate D-PBS (Without Ca++ and Mg++).
3. Add 2.5 mL of ACF Enzymatic Dissociation Solution. Incubate at 37°C for 7 - 8 minutes, until cells can be dislodged with gentle tapping of the flask.
4. Add 2.5 mL of ACF Enzyme Inhibition Solution and wash over the inner flask surface. Transfer entire cell suspension to a 15 mL conical tube.
5. Centrifuge cell suspension at 314 x *g* for 5 minutes. Aspirate supernatant.
6. Add 1 - 2 mL of PneumaCult™-Ex Plus Medium to resuspend the cell pellet.
7. Perform a viable cell count using Trypan Blue and a hemocytometer.
8. To a T-25 cm^2 flask containing PneumaCult™-Ex Plus Medium (room temperature), add enough cell suspension to seed 1.25 x 10^5 live cells. Gently shake the flask, then incubate at 37°C and 5% CO_2. Reserve the remaining cell suspension for initiation of 3D HBEC expansion (section C step 4). Proceed to section C.
9. Perform a full-medium change on the T-25 cm^2 flask every 2 days. When the flask is 50 - 60% confluent, repeat steps 1 - 7.

C. 3D HBEC Expansion in PneumaCult™ Airway Organoid Seeding Medium

1. Place a 15 mL conical tube on ice for 5 - 10 minutes.
2. Calculate the volumes of Matrigel® and PneumaCult™ Airway Organoid Seeding Medium required for plating the desired number of 50 µL domes, as described below. Each dome will be 90% Matrigel® and 10% PneumaCult™ Airway Organoid Seeding Medium.
   a. Calculate the total volume (V_{total}) as follows: Number of domes x 50 µL x 1.1 (prepare 10% extra volume)

b. Calculate the volume of Matrigel® as follows: V_{total} x 0.9

c. Calculate the volume of Seeding Medium as follows: V_{total} x 0.1

3. Add the calculated volumes of Matrigel® and Seeding Medium to the cooled 15 mL tube. Mix thoroughly by pipetting up and down 10 - 15 times. Avoid introducing bubbles. Place tube on ice.
4. To the Matrigel®-medium mixture, add 2 x 10^3 HBECs (reserved in section B step 8) per 50 µL. Mix thoroughly by pipetting up and down 10 times. Avoid introducing bubbles. Place tube on ice.

   NOTE: A seeding density of 2 x 10^3 HBECs per 50 µL is based on P3 HBECs (starting with P2 HBECs in section A and performing one passage); optimize seeding density to best fit user requirements. A higher seeding density with higher passage numbers often improves differentiation.
5. Remove the 24-well plate from the incubator and 200 µL pipette tips from the fridge and place in a biosafety cabinet.
6. Using a cooled 200 µL pipette tip, draw up 50 µL of the Matrigel®-medium-cell suspension and add to one of the 8 central wells of the warm 24-well plate as follows:

   a. Hold the pipette vertically over the center of the well, and bring the tip near to, but not in contact with, the floor of the well.

   b. Gently dispense (to the first stop on the pipette) the suspension while lifting the pipette away from the well.

   NOTE: Work quickly to plate the Matrigel®-medium-cell suspension within ~60 seconds of removing it from the ice. The 8 wells in the center of the plate are most suitable for domes since their surfaces are the most even. Wells at the edges of the plate are often slightly slanted, resulting in domes touching the side of the well and flattening.

   c. Repeat steps a & b for each dome to be plated.
7. Carefully transfer the plate to a 37°C incubator. Incubate for 15 minutes to allow domes to solidify. Do not disturb the domes.
8. Remove the plate from the incubator and place in the biosafety cabinet.
9. Add 500 - 750 µL of PneumaCult™ Airway Organoid Seeding Medium to each well containing a dome by pipetting the medium gently down the side of the well. Do not pipette directly onto the domes.
10. Incubate at 37°C and 5% CO_2.
11. Perform a full-medium change every 2 days by carefully aspirating the medium and adding 500 - 750 µL of room temperature PneumaCult™ Airway Organoid Seeding Medium. After 4 - 7 days of incubation (optimize this duration to best fit user requirements), proceed to section D for initiation of 3D differentiation.

    NOTE: For weekends, change the medium on Friday afternoon with 1 mL PneumaCult™ Airway Organoid Seeding Medium, then first thing on Monday morning with 500 - 750 µL of medium.

D. 3D HBEC Differentiation in PneumaCult™ Airway ODM

1. Prepare PneumaCult™ Airway ODM and warm to room temperature (15 - 25°C).
2. Carefully aspirate medium from the domes of 3D HBEC cultures. Add 750 - 1000 µL of PneumaCult™ Airway ODM to each well by pipetting the medium gently down the side of the well. Do not pipette directly onto the domes.
3. Incubate at 37°C and 5% CO_2.
4. Perform a full-medium change every 2 days by carefully aspirating the medium and adding 750 - 1000 µL of room temperature PneumaCult™ Airway ODM. Incubate at 37°C for 21 - 28 days.

   NOTE: On weekends, change the medium on Friday afternoon with 1 mL PneumaCult™ Airway ODM, then first thing on Monday morning with 750 - 1000 µL medium.

   NOTE: Signs of differentiation will become visible after approximately 14 days of culture in PneumaCult™ Airway ODM, including presence of a central lumen and differentiated cell types, notably ciliated cells and goblet cells. Organoids are usually fully differentiated after 21 - 28 days, at which point characterization assays may be performed. Organoids cultured in PneumaCult™ Airway ODM are not suitable for passaging.