PneumaCult™ Apical-Out Airway Organoid Medium

Serum- and BPE-free medium for differentiation of human primary bronchial epithelial cells or human airway epithelial cells to mature apical-out airway organoids

Catalog #100-0620 1 Kit
Catalog #100-0746 1 Kit
Catalog #100-0747 1 Kit

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Product Description

PneumaCult™ Apical-Out Airway Organoid (AOAO) Medium is a serum- and BPE-free medium for the differentiation of human primary bronchial epithelial cells (HBECs) or human airway epithelial cells (HAECs) to mature apical-out airway organoids in 15 days. When fully differentiated, organoids exhibit outward-facing ciliated cells characteristic of the apical side of the epithelium lining the airways in vivo. Culture of HBECs or HAECs in PneumaCult™-Ex Plus Medium (Catalog #05040) prior to inducing organoid differentiation with PneumaCult™ AOAO Medium constitutes a complete serum- and BPE-free workflow. PneumaCult™ AOAO Medium is also available in a kit that includes one AggreWell™400 24-well plate (Catalog #100-0746) or 5x AggreWell™400 24-well plates (Catalog #100-0747). Applications of apical-out airway organoids include infectious disease modeling and high-throughput drug screening in in vitro, three-dimensional (3D) model systems.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	KIT COMPONENTS
PneumaCult™ Apical-Out Airway Organoid Medium	100-0620	1 Kit	• PneumaCult™ Apical-Out Airway Organoid Basal Medium • PneumaCult™ Apical-Out Airway Organoid 10X Supplement • PneumaCult™ Apical-Out Airway Organoid 1000X Supplement
PneumaCult™ Apical-Out Airway Organoid Medium with AggreWell™400 (24-well plate)	100-0746	1 Kit	• PneumaCult™ Apical-Out Airway Organoid Medium (Catalog #100-0620) • AggreWell™400 24-well Plate, 1 Plate (Catalog #34411)
PneumaCult™ Apical-Out Airway Organoid Medium with AggreWell™400 (5 x 24-well plates)	100-0747	1 Kit	• PneumaCult™ Apical-Out Airway Organoid Medium (Catalog #100-0620) • AggreWell™400 24-well Plate, 5 Plates (Catalog #34415)

Component Storage and Stability

The following components are sold as a complete kit (Catalog #100-0620) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™ Apical-Out Airway Organoid Basal Medium	100-0621	450 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
PneumaCult™ Apical-Out Airway Organoid 10X Supplement*	100-0622	50 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
PneumaCult™ Apical-Out Airway Organoid 1000X Supplement**†	100-0623	500 µL	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.
** Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in dimethyl sulfoxide (DMSO). DMSO is a strong solvent and skin penetrant and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.
† This component is light sensitive; minimize exposure to light when handling.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Anti-Adherence Rinsing Solution	07010
DMEM/F-12 with 15 mM HEPES	36254
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
D-PBS (Without Ca++ and Mg++)	37350
AggreWell™400 24-well plate, 1 plate* OR AggreWell™400 24-well plate, 5 plate**	34411 OR 34415
Tissue culture-treated 24-well flat-bottom plate	e.g. 38017
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Conical tubes	e.g. 38009
Trypan Blue	07050

* Included in PneumaCult™ Apical-Out Airway Organoid Medium with AggreWell™400 (24-well plate; Catalog #100-0746)
** Included in PneumaCult™ Apical-Out Airway Organoid Medium with AggreWell™400 (5 x 24-well plates; Catalog #100-0747)

Preparation of Reagents and Materials

Use sterile technique when preparing the following materials and media.

A. Complete PneumaCult™ Apical-Out Airway Organoid (AOAO) Medium

The following example is for preparing 10 mL of complete PneumaCult™ AOAO Medium (PneumaCult™ Apical-Out Airway Organoid Basal Medium + PneumaCult™ Apical-Out Airway Organoid 10X Supplement + PneumaCult™ Apical-Out Airway Organoid 1000X Supplement + Heparin Solution + Hydrocortisone Stock Solution). If preparing other volumes, adjust accordingly.

1. Thaw PneumaCult™ Apical-Out Airway Organoid 10X Supplement at 2 - 8°C overnight. Mix the 10X Supplement gently by inverting the vial; do not vortex. Thaw PneumaCult™ Apical-Out Airway Organoid 1000X Supplement at room temperature (15 - 25°C). Mix the 1000X Supplement thoroughly; do not vortex.

   NOTE: Once thawed, use immediately or aliquot and store supplements at -20°C. Do not exceed the shelf life of the supplements. After thawing the aliquoted supplements, use immediately. Do not re-freeze.
2. Combine the following:
   - 8.92 mL PneumaCult™ Apical-Out Airway Organoid Basal Medium
   - 1 mL PneumaCult™ Apical-Out Airway Organoid 10X Supplement
   - 10 µL PneumaCult™ Apical-Out Airway Organoid 1000X Supplement
   - 20 µL Heparin Solution
   - 50 µL Hydrocortisone Stock Solution

   Mix thoroughly; do not vortex.

   NOTE: If not used immediately, store complete PneumaCult™ AOAO Medium at 2 - 8°C for up to 2 weeks. Do not freeze.

B. Pre-Treating Plates

NOTE: Pre-treating plates with Anti-Adherence Rinsing Solution prevents cell adhesion and promotes efficient aggregate formation.

Using sterile technique, pre-treat the AggreWell™ 24-well plate (for section A of Directions for Use) and the tissue culture-treated 24-well flat-bottom plate (for section B of Directions for Use) as follows:

1. Open the AggreWell™400 24-well plate and the 24-well tissue culture-treated flat-bottom plate in a biosafety cabinet.

   NOTE: Do not expose the plates to organic solvents, including ethanol and isopropanol.
2. Pre-treat wells with Anti-Adherence Rinsing Solution as follows:
   a. Add 500 µL Anti-Adherence Rinsing Solution to each well to be used.

b. Swirl the cultureware to spread the solution evenly across the surface and the walls of the wells.

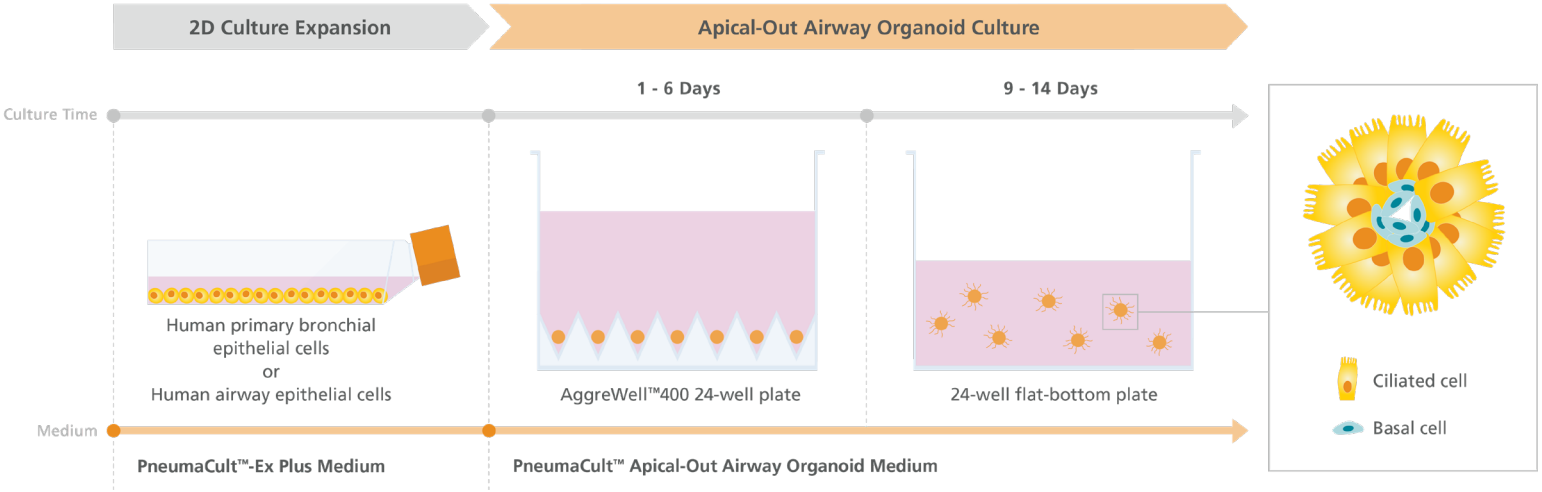
c. Centrifuge the plate at 1300 x *g* for 10 minutes.

NOTE: Plates must be well balanced. Prepare balance plates using standard plates filled with water to match the weight and position of the 24-well plates.

d. Remove Anti-Adherence Rinsing Solution from each well using an aspirator or a 1 mL pipettor.

3. Rinse each well with 1 mL of room temperature (15 - 25°C) DMEM/F-12 with 15 mM HEPES (DMEM/F-12).
4. Fill each washed well with 0.5 mL of DMEM/F-12 and store the plate at 37°C and 5% CO_2. Treated plates can be stored for at least 5 days, but ideally should be used on the same day. Remove DMEM/F-12 immediately before use. Do not let the plate dry before use.

Protocol Diagram



Directions for Use

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

A. Aggregation of HBECs or HAECs

B. Differentiation to Apical-Out Airway Organoids

A. Aggregation of HBECs or HAECs

The following example is for passaging human primary bronchial epithelial cells (HBECs) or human airway epithelial cells (HAECs) from a T-25 cm^2 flask and plating them into a single well of an AggreWell™400 24-well plate for aggregation. If using more wells, adjust volumes accordingly.

NOTE: Donor optimization is recommended before performing this assay on a larger scale. Refer to the Donor Optimization section for instructions on optimizing aggregation time for each donor.

1. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™ AOAO Medium (Preparation section A), ACF Enzymatic Dissociation Solution, and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C).
2. Pre-treat an AggreWell™400 24-well plate with Anti-Adherence Rinsing Solution (Preparation section B).
3. Wash HBECs or HAECs in a T-25 cm^2 flask with 5 mL D-PBS (Without Ca++ and Mg++). Remove D-PBS.
4. Add 2.5 mL ACF Enzymatic Dissociation Solution. Incubate at 37°C for 6 - 8 minutes, until cells can be dislodged with gentle tapping of the flask.
5. Add 2.5 mL ACF Enzyme Inhibition Solution, then transfer cells to a 15 mL conical tube.
6. Centrifuge cell suspension at 350 x *g* for 5 minutes.
7. Discard the supernatant and resuspend the cell pellet in 1 - 2 mL complete PneumaCult™ AOAO Medium.

   NOTE: HBECs or HAECs suspended in complete PneumaCult™ AOAO Medium can be used for expansion in tissue culture flasks with PneumaCult™-Ex Plus Medium (Catalog #05040) or PneumaCult™-Ex Medium (Catalog #05008) by centrifuging to remove PneumaCult™ AOAO Medium and resuspending the cells in the appropriate expansion medium.
8. Perform a viable cell count using Trypan Blue and a hemocytometer.
9. Add complete PneumaCult™ AOAO Medium to the AggreWell™400 well (prepared in step 2) to reach a total volume of 1 mL, then transfer 120,000 cells to the well. Mix the suspension thoroughly.

10. Centrifuge the AggreWell™400 plate at 100 x *g* for 3 minutes.
11. Incubate at 37°C and 5% CO_2 for 24 hours - 6 days to aggregate cells.

    NOTE: Cells from most donors require 24 hours of aggregation. Do not incubate for longer than required; additional time in aggregation might impact the quality of the cultures. For adaptation of this step to each donor, refer to section B step 5.

    NOTE: If aggregation for more than 2 days is required, perform a partial-medium change on Day 2 by removing 0.5 mL of medium along the wall of the well. Slowly add 0.5 mL complete PneumaCult™ AOAO Medium, taking care not to suspend the aggregates. Continue partial-medium changes every other day.

B. Differentiation to Apical-Out Airway Organoids

1. Pre-treat a tissue culture-treated 24-well flat-bottom plate with Anti-Adherence Rinsing Solution (Preparation section B).
2. Warm a sufficient volume of complete PneumaCult™ AOAO Medium to room temperature (15 - 25°C).
3. Add 1 mL complete PneumaCult™ AOAO Medium to the AggreWell™ well containing aggregates. Using a 1 mL pipettor, mix the medium to dislodge the aggregates from the microwell.
4. Transfer 1 mL of the aggregate suspension to the pre-treated 24-well flat-bottom plate (prepared in step 1). Two wells should be generated from each AggreWell™ well. Incubate at 37°C and 5% CO_2 for 24 hours.
5. Observe the aggregates under the microscope:
   - If no fusion between aggregates is observed (Figure 1B), proceed to step 6.
   - If fusion between aggregates is observed (Figure 1A), restart the culture and increase the aggregation time until fusion between aggregates is not observed.

   NOTE: To efficiently optimize donor aggregation duration, a pilot experiment can be performed as described in the Donor Optimization section at the end of this protocol.
6. Perform a partial-medium change as follows:
   a. Tilt the plate at a 25- to 30-degree angle and remove 0.5 mL of medium along the wall of the well, taking care not to remove suspended organoids.

      NOTE: A dark surface placed at the bottom of the plate can help to create the necessary contrast to better visualize the organoids.

   b. Add 0.5 mL complete PneumaCult™ AOAO Medium.
   c. Incubate at 37°C and 5% CO_2.
7. Perform a partial-medium change every 2 days. Apical-out airway organoids should display beating cilia by day 15 (9 - 14 days in the 24-well flat-bottom plate), at which point downstream assays may be performed.

   IMPORTANT: If downstream applications include washing steps, perform all organoid washes in DMEM/F-12 warmed to room temperature. Washing apical-out airway organoids with D-PBS might have a detrimental effect on the structure of the organoids.

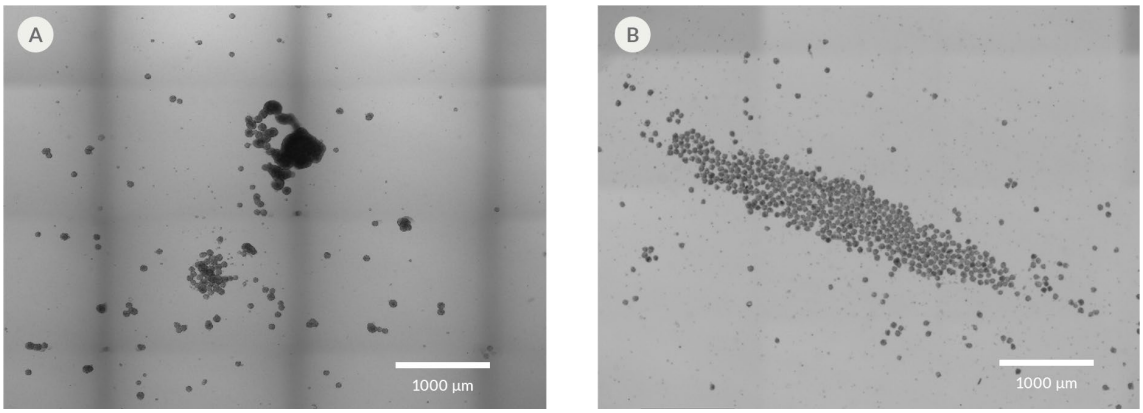


FIGURE 1. Fusion vs. No Fusion of Apical-Out Airway Organoids in Suspension Culture

Representative images of organoid cultures in (A) non-optimized and (B) optimized aggregate duration conditions.

(A) Aggregates suspended after insufficient aggregation time in AggreWell™400. These aggregates tend to fuse together and generate large cell masses, which decreases the final number of organoids in culture.

(B) Aggregates suspended after optimal aggregation time in AggreWell™400. Optimized aggregates do not fuse together in suspension, which maximizes the efficiency of the assay.

Assessment of Apical-Out Airway Organoids

Assessment of apical-out airway organoids can be verified by immunocytochemistry staining (e.g. monoclonal anti-acetylated tubulin antibody, clone 6-11B-1). Results may vary depending on cell line used.

Donor Optimization

Due to differences in aggregation time required between donors, it is recommended to first optimize the assay for each donor in order to maximize efficiency. The following procedure is an example for optimizing the generation of apical-out airway organoids using complete PneumaCult™ AOAO Medium for one new donor.

1. Perform steps 1 - 9 in section A of Directions for Use, adding cells to at least 6 wells of an AggreWell™400 24-well plate in step 9.
2. Centrifuge the AggreWell™400 plate at 100 x *g* for 3 minutes.
3. Incubate the plate at 37°C and 5% CO_2 for 24 hours.
4. Pre-treat a tissue culture-treated 24-well flat-bottom plate with Anti-Adherence Rinsing Solution (Preparation section B).
5. Select one well of the AggreWell™ plate containing aggregates, and add 1 mL of complete PneumaCult™ AOAO Medium. Using a 1 mL pipettor, mix the medium to dislodge the aggregates from the microwell.
6. Transfer 1 mL of the aggregate suspension to the flat-bottom plate (prepared in step 4). Two wells should be generated from each AggreWell™400 well.
7. Incubate the flat-bottom plate and the AggreWell™400 plate at 37°C and 5% CO_2 for 24 hours.
8. Observe the flat-bottom plate under the microscope for the presence of large aggregates.
9. If large aggregates have formed, follow steps 5 - 8 of this section for another AggreWell™400 well.
10. Repeat this transfer procedure (aggregates from AggreWell™400 wells to the flat-bottom plate) until no fusion between aggregates is observed. The aggregation time in AggreWell™ where no aggregate fusion was observed in the 24 hours after suspension should be used in future experiments using the donor tested.