PneumaCult™ Apical-Out Airway Organoid Secretory Medium

Serum- and BPE-free medium for the generation of physiologically representative apical-out airway organoids

Catalog #100-2078 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

PneumaCult™ Apical-Out Airway Organoid Secretory (AOAOS) Medium is a two-stage, serum- and bovine pituitary extract (BPE)-free medium for differentiating human primary bronchial epithelial cells (HBECs) or human airway epithelial cells (HAECs) to mature, physiologically representative apical-out airway organoids. These organoids contain basal, secretory (goblet), and 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) or PneumaCult™-NGEx Medium (Catalog #100-1505) prior to inducing organoid differentiation with PneumaCult™ AOAOS Medium constitutes a complete serum- and BPE-free workflow. Apical-out airway organoids generated using PneumaCult™ AOAOS Medium can be used for infectious disease modeling and high-throughput drug screening in three-dimensional (3D) in vitro systems.

Component Storage and Stability

The following components are sold as a complete kit (Catalog #100-2078); PneumaCult™ Apical-Out Airway Organoid Secretory Supplement (Catalog #100-1780) is also 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.
PneumaCult™ Apical-Out Airway Organoid Secretory Supplement†	100-1780	3 x 1 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.

** 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. Use extra caution when handling this product.

† This component is light sensitive; minimize exposure to light when handling.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
AggreWell™400 24-well plate, 1 plate OR AggreWell™400 24-well plate, 5 plates	34411 OR 34415
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Anti-Adherence Rinsing Solution	07010
Conical tubes, 15 mL	e.g. 38009
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
Tissue culture-treated 24-well flat-bottom plate	e.g. 38017
Tissue culture-treated T-25 cm^2 flask, with vented cap	e.g. Corning 353109
Trypan Blue	07050

Preparation of Reagents and Materials

Use sterile technique when preparing the following materials and media.

A. Complete PneumaCult™ Apical-Out Airway Organoid Secretory (AOAOS) Medium 1 and Medium 2

The following example is for preparing 10 mL of complete AOAOS Medium 1 (Basal Medium + 10X Supplement + 1000X Supplement + Heparin Solution + Hydrocortisone Stock Solution) and 10 mL of complete AOAOS Medium 2 (Basal Medium + 10X Supplement + Secretory 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™ Airway Organoid 1000X Supplement and PneumaCult™ Airway Organoid Secretory Supplement at room temperature (15 - 25°C). Mix the supplements 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. Prepare media by combining components as indicated in Table 1.

Table 1. Preparation of AOAOS Medium 1 and Medium 2

MEDIUM	COMPONENT NAME	VOLUME	PREPARATION AND STORAGE
Medium 1 (10 mL)	PneumaCult™ Apical-Out Airway Organoid Basal Medium	8.92 mL	Mix thoroughly. If not using immediately, store at 2 - 8°C for up to 2 weeks. Do not freeze.
	PneumaCult™ Apical-Out Airway Organoid 10X Supplement	1 mL	
	PneumaCult™ Apical-Out Airway Organoid 1000X Supplement	10 µL	
	Heparin Solution	20 µL	
	Hydrocortisone Stock Solution	50 µL	
Medium 2 (10 mL)	PneumaCult™ Apical-Out Airway Organoid Basal Medium	8.83 mL	Mix thoroughly. If not using immediately, store at 2 - 8°C for up to 2 weeks. Do not freeze.
	PneumaCult™ Apical-Out Airway Organoid 10X Supplement	1 mL	
	PneumaCult™ Apical-Out Airway Organoid Secretory Supplement	100 µL	
	Heparin Solution	20 µL	
	Hydrocortisone Stock Solution	50 µL	

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 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, they should be used on the same day. Remove DMEM/F-12 immediately before use. Do not let the plate dry before use.

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

I. Using HBECs or HAECs Expanded in PneumaCult™-NGEx Medium

II. Using HBECs or HAECs Expanded in PneumaCult™-Ex Plus Medium

A. Aggregation of HBECs or HAECs

The following example is for passaging HBECs or 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 when using cells expanded in PneumaCult™-Ex Plus Medium 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™ AOAOS Medium 1 (Preparation of Reagents and Materials 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 of Reagents and Materials 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 and 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™ AOAOS Medium 1.

   NOTE: HBECs or HAECs suspended in complete PneumaCult™ AOAOS Medium 1 and not required for differentiation can be used for further expansion in tissue culture flasks (e.g. Corning Catalog #353109) with PneumaCult™-NGEx Medium (Catalog #100-1505) or PneumaCult™-Ex Plus Medium (Catalog #05040); perform this expansion by centrifuging to remove PneumaCult™ AOAOS Medium 1 and resuspending the cells in the appropriate expansion medium.
8. Perform a viable cell count using Trypan Blue and a hemocytometer (e.g. Catalog #100-1181).
9. Add complete PneumaCult™ AOAOS Medium 1 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. If using cells expanded in PneumaCult™-NGEx Medium, proceed to Directions for Use section B, subsection I. If using cells expanded in PneumaCult™-Ex Plus Medium, proceed to Directions for Use section B, subsection II.

B. Differentiation to Apical-Out Airway Organoids

The following protocols (I and II) are for differentiation to apical-out airway organoids using HBECs or HAECs expanded in either PneumaCult™-NGEx Medium (I) or PneumaCult™-Ex Plus Medium (II).

I. Using HBECs or HAECs Expanded in PneumaCult™-NGEx Medium

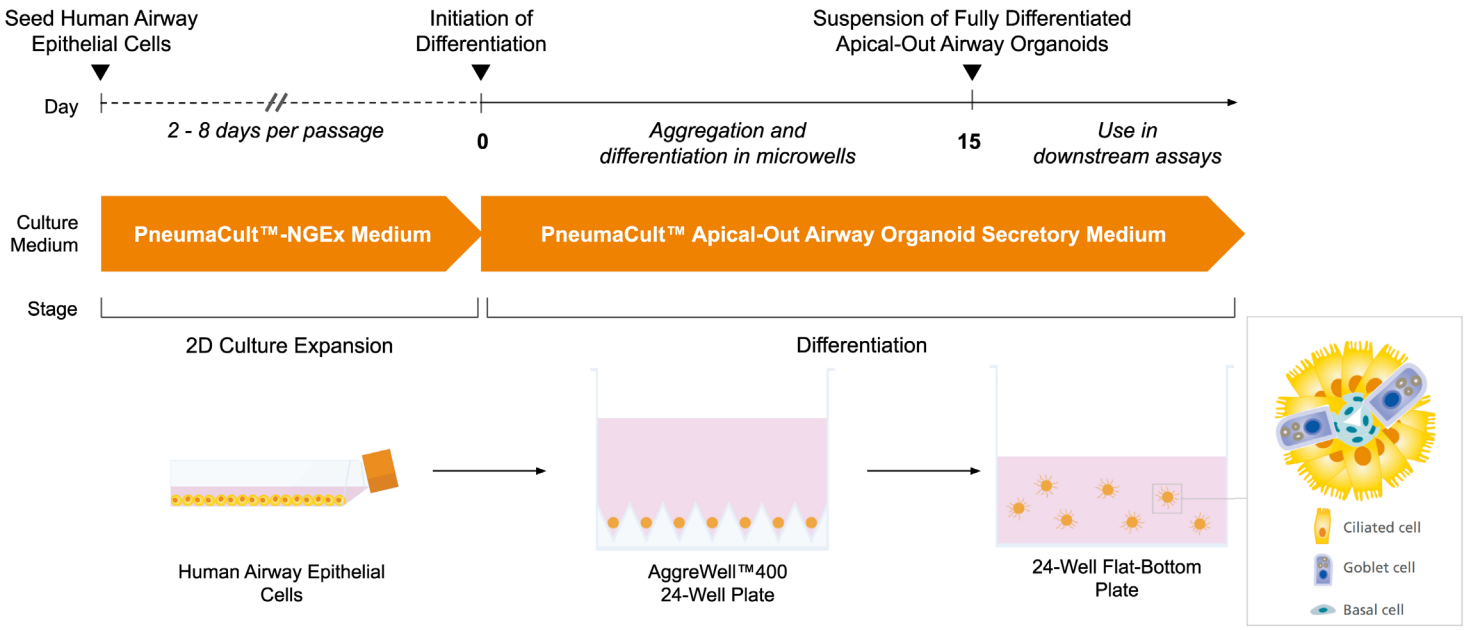

Figure 1. Protocol Diagram for the Generation of Apical-Out Airway Secretory Organoids Using Cells Expanded in PneumaCult™-NGEx Medium

1. On Day 2, warm a sufficient volume of complete PneumaCult™ AOAOS Medium 1 to 37°C. Perform a partial-medium change by removing 0.5 mL of medium along the wall of the well. Slowly add 0.5 mL of complete PneumaCult™ AOAOS Medium 1, taking care not to suspend the aggregates. Continue partial-medium changes every other day.
2. On Day 6, warm a sufficient volume of complete PneumaCult™ AOAOS Medium 2 to 37°C. Then, remove 0.8 mL of medium along the wall of the well. Slowly add 1 mL of complete PneumaCult™ AOAOS Medium 2, taking care not to suspend the aggregates.

   NOTE: Using an automated pipettor is recommended to minimize aggregate disruption. If an automated pipettor is not available and significant dislodging (more than one-fifth of the well) is detected, instead perform two sequential partial-medium changes; for each partial-medium change, remove 0.5 mL of medium alongside the well and replace it with 0.5 mL of fresh PneumaCult™ AOAOS Medium 2 warmed to 37°C.
3. Perform a partial-medium change every 2 days using PneumaCult™ AOAOS Medium 2 warmed to 37°C. Apical-out airway organoids should have beating cilia and secretory cells by Day 15; at this point, downstream assays may be performed.

   NOTE: Beating cilia can usually be observed from Day 10 onwards.
4. To perform downstream assays, carefully dislodge organoids by pipetting with a 1 mL pipettor, and transfer to a flat-bottom plate treated with Anti-Adherence Rinsing Solution (Preparation of Reagents and Materials section B).

   NOTE: If the assay includes washing the organoids, perform all washing steps 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.

II. Using HBECs or HAECs expanded in PneumaCult™-Ex Plus Medium

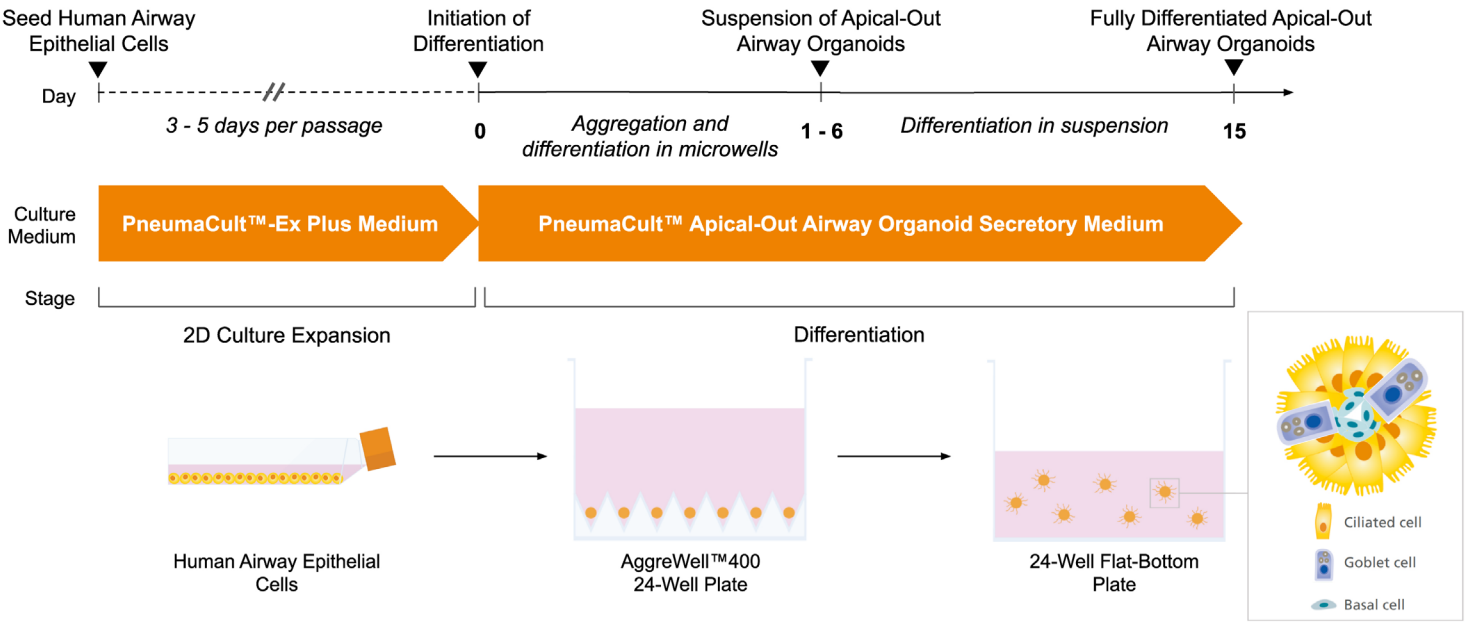

Figure 2. Protocol Diagram for the Generation of Apical-Out Airway Secretory Organoids Using Cells Expanded in PneumaCult™-Ex Plus Medium

1. Incubate the AggreWell™400 plate at 37°C and 5% CO_2 for 24 hours - 6 days.

   NOTE: Cells from most donors require 24 hours of aggregation. To adapt this step to each donor, refer to the Donor Optimization section.

   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 of complete PneumaCult™ AOAOS Medium 1, taking care not to suspend the aggregates. Continue partial-medium changes every other day.
2. Pre-treat a tissue culture-treated 24-well flat-bottom plate with Anti-Adherence Rinsing Solution (Preparation of Reagents and Materials section B).
3. Warm a sufficient volume of complete PneumaCult™ AOAOS Medium 1 to room temperature (15 - 25°C).
4. Add 1 mL complete PneumaCult™ AOAOS Medium 1 to the AggreWell™ well containing aggregates. Using a 1 mL pipettor, mix the medium to dislodge the aggregates from the microwell.
5. Transfer 1 mL of the aggregate suspension to the pre-treated 24-well flat-bottom plate (prepared in step 2). Two wells should be generated from each AggreWell™ well. Incubate at 37°C and 5% CO_2 for 24 hours.

6. Observe the aggregates under the microscope:
   - If fusion between aggregates is observed (Figure 3A), restart the culture and increase the aggregation time until fusion between aggregates is minimal.

     NOTE: To determine the optimal aggregation period for each donor, refer to the Donor Optimization section.
   - If no fusion between aggregates is observed (Figure 3B), proceed to step 7.
7. 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 of complete PneumaCult™ AOAOS Medium 1.
   c. Incubate at 37°C and 5% CO_2.
8. Perform a partial-medium change on Day 4 using complete PneumaCult™ AOAOS Medium 1. On Day 6, follow step 7a and remove 0.8 mL of medium. Add 1 mL of complete PneumaCult™ AOAOS Medium 2.
9. Perform a partial-medium change every 2 days using complete PneumaCult™ AOAOS Medium 2. Apical-out airway organoids should have beating cilia and secretory cells by Day 15 (9 - 14 days in the 24-well flat-bottom plate), at which point characterization assays may be performed.

   IMPORTANT: If downstream applications include washing the organoids, perform all washing steps 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.

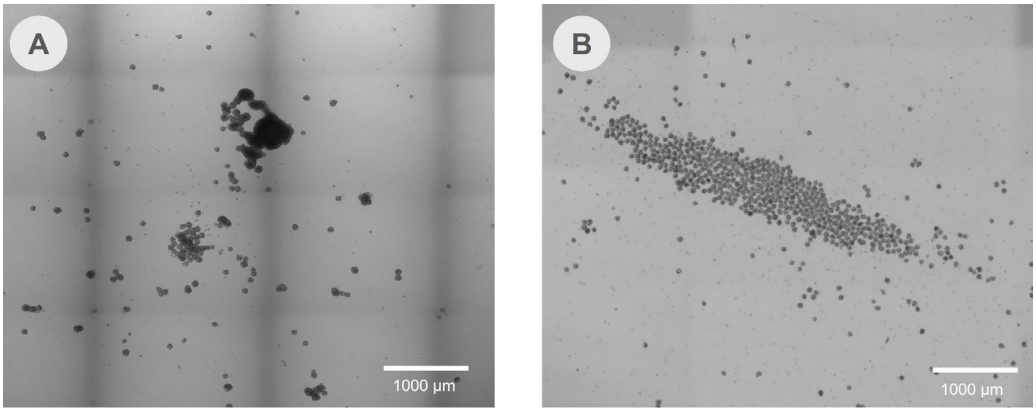

Figure 3. Fusion vs. No Fusion of Apical-Out Airway Organoids in Suspension Culture

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 and passage of HBECs or HAECs 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 the efficiency. The following procedure is an example for optimizing the generation of apical-out airway organoids using complete PneumaCult™ AOAOS Medium 1 for one new donor.

NOTE: This procedure is necessary only when using cells expanded in PneumaCult™-Ex Plus Medium.

1. Perform steps 1 - 10 of section A of Directions for Use, adding cells to at least 6 wells of an AggreWell™400 24-well plate in step 9.
2. Incubate the plate at 37°C and 5% CO_2 for 24 hours.
3. Pre-treat a tissue culture-treated 24-well flat-bottom plate with Anti-Adherence Rinsing Solution (Preparation of Reagents and Materials section B).

4. Select one well of the AggreWell™400 plate containing aggregates, and add 1 mL of complete PneumaCult™ AOAOS Medium 1. Using a 1 mL pipettor, mix the medium to dislodge the aggregates from the microwell.
5. 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.
6. Incubate the flat-bottom plate and the AggreWell™400 plate at 37°C and 5% CO_2 for 24 hours.
7. Observe the flat-bottom plate under the microscope for the presence of large aggregates indicative of fusion (Figure 3A).
8. If large aggregates have formed, follow steps 4 - 7 of this section for another AggreWell™400 well.
9. Repeat this daily 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.