Data Sheet

3dGRO® Human Lung Organoids (LPTO.245)

Stem Cell Line

SCC599

Pack Size: ≥ 1500 organoids/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Patient derived organoids (PDOs) are novel *in vitro* 3D cell models that preserve the original tissue physiology and molecular pathology, thus representing a clinically relevant alternative to traditional 2D cell lines and an effective tool to refine and reduce animal models. Adult tissue derived organoids are phenotypically and genetically stable in long term culture, presenting more mature phenotypes compared to iPSC-derived organoids. Importantly, PDOs have shown to be able to predict patient clinical responses to chemotherapeutics.

Lung cancer is the leading cause of cancer cell death and non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancers. There are 3 main subtypes of NSCLC:

- Adenocarcinoma (40% of lung cancers) are typically found in the mucus-secreting cells of the lung.
- Squamous cell carcinoma (25-30% of lung cancers) occurs in the flat cells that line the inside of the lung airways.
- Large cell carcinoma (10-15% of lung cancers) appears large and round and can occur in any part of the lung and tends to grow and spread faster than adenocarcinoma or squamous cell carcinoma.

We offer a portfolio of human lung cancer organoids containing 7 adenocarcinoma (SCC599, SCC600, SCC601 through SCC606) and 2 squamous cell carcinoma (SCC607, SCC608) organoid lines derived from individuals with adenocarcinoma and squamous cell carcinoma, respectively. 3dGRO® Human Lung Organoids are derived from patient-derived xenograft, primary tumors or metastatic patient tissues and express the major epithelial markers including pan-cytokeratin and EPCAM along with the alveolar type 2 (AT2) cell markers-surfactant proteins SFTPB and SFTPC and the ciliated cell marker acetyl-α-tubulin. Adenocarcinoma organoid lines express the key lung adenocarcinoma markers including thyroid transcription factor 1 (TTF-1), cytokeratin 7 (CK7) and airway goblet cell marker MUC5AC and do not express the squamous cell carcinoma markers such as P63 and CK5/6. Squamous cell carcinoma organoid lines exhibit distinct squamous cell carcinoma markers including P63 and CK5/6, and do not express the adenocarcinoma markers cytokeratin-7 (CK7) and TTF-1.

3dGRO® Organoids were derived utilizing HUB Organoid Technology. The purchaser of this product shall agree to HUB's terms of use (listed in Academic Use Agreement), which shall be separately acknowledged and accepted by such purchaser, prior to transfer of this product to purchaser.

Organoid Line Characteristics

- Sex: Female
- Age: 69 Years
- Organ: Lung (PDX-Acinar/Solid)
- Disease: Adenocarcinoma

Quality Control Testing

- Viability: ≥ 1500 organoids/vial
- Organoid Growth: Pass
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services
- Mycoplasma Contamination: Negative
- STR Profile: Pass

Storage and Handling

Store in liquid nitrogen. The organoids can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

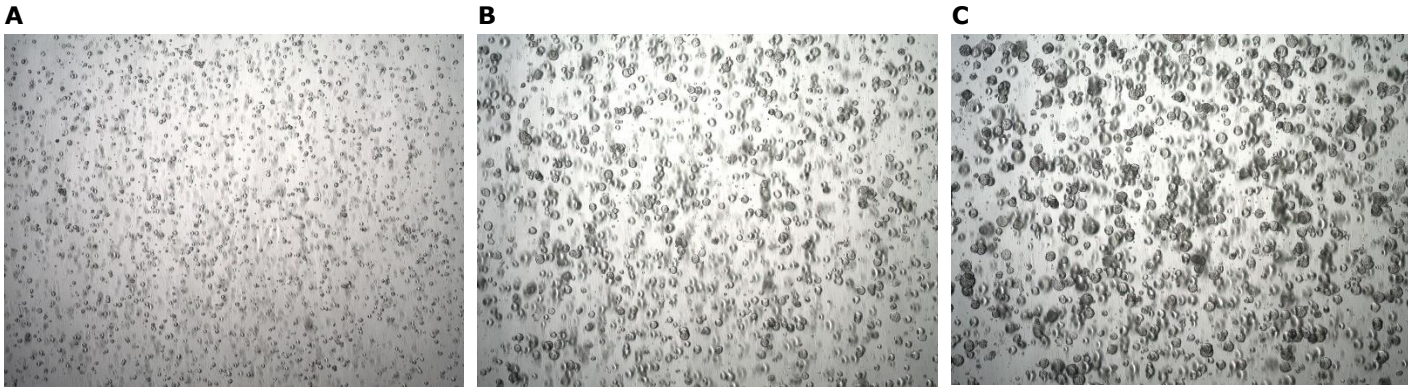

Figure 1: LPT.245 lung organoids cultured over 9 days (**A, B, C**) show an overall increase in size and maintain rounded morphology.

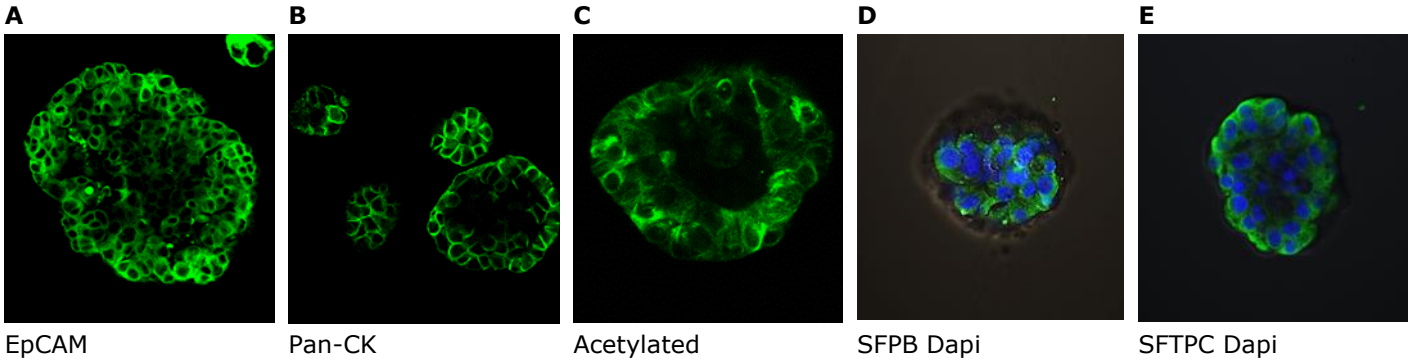

Figure 2: Immunocytochemical (ICC) characterization of human lung cancer organoids. Lung cancer organoids are positive for EpCAM (**A**), pan-cytokeratin (**B**), acetylated α-tubulin (**C**), surfactant protein-B (**D**, SFTPB), and surfactant protein-C (**E,** SFTPC). Nuclear staining with Dapi (blue).

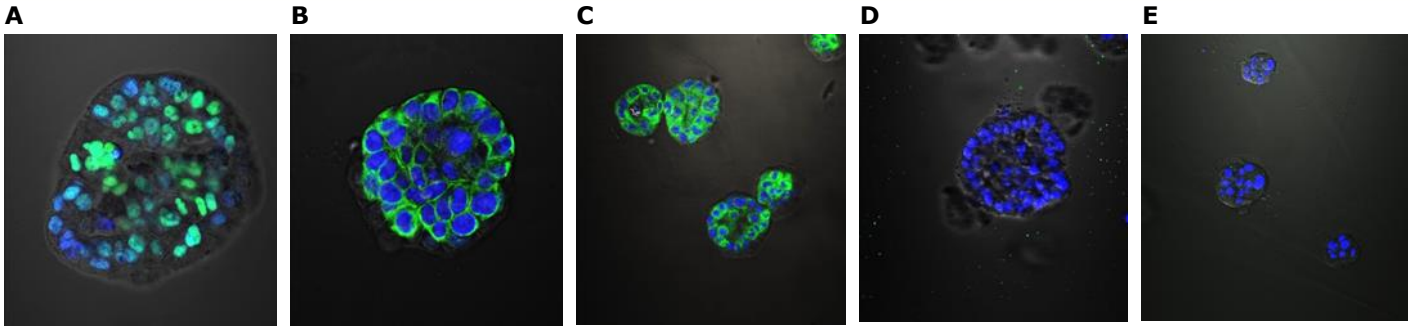

Figure 3: Lung adenocarcinoma organoid lines (SCC599, SCC600, SCC601-SCC606) express the key lung adenocarcinoma markers including thyroid transcription factor 1 (TTF-1), cytokeratin 7 (CK7) and the airway goblet cell marker MUC5AC, but do not express the squamous cell carcinoma markers p63 and CK5/6.

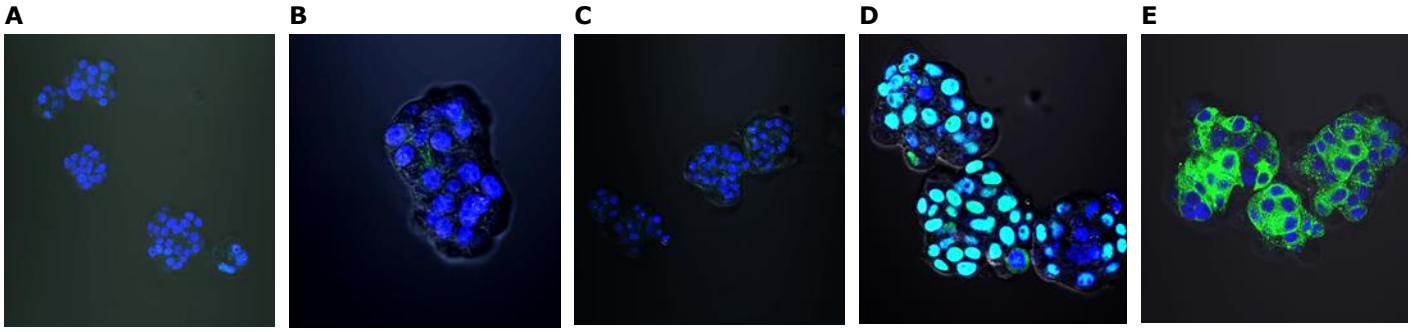

Figure 4: Lung squamous cell carcinoma organoid lines (SCC607 and SCC608) do not express the adenocarcinoma markers TTF-1, CK7 and MUC5AC, but do express p63 and CK5, two characteristic markers of squamous cell carcinoma tissues.

Protocols

All protocols are performed within a Class II laminar flow biohood and with an aspirator unless otherwise specified. Incubators are humidified and are set to 37 °C and 5% CO_2. PPE should be worn-gloves, lab coat and safety glasses.

Preparing 1X Complete Medium for LPTO.245 Lung Organoids.

1. To make 500 mL of complete culture media, combine the following components:

Components	Quantity	Final Conc.
DMEM/F12 PLUS Basal Medium	412.2 mL	
Ala-Glu (100X), 200 mM	5 mL	2 mM
HEPES Solution, 1 M in water	5 mL	10 mM
B-27™ Supplement (50X), serum free	10 mL	1X
Nicotinamide, 1 M in water	10 mL	10 mM
N-Acetyl-L-cysteine, prepared as 500 mM solution in water	1.25 mL	1.25 mM
Recombinant Human FGF-7, reconstituted to 100 µg/mL in PBS/ 0.1% BSA	200 µL	40 ng/mL
Recombinant Human FGF-10, reconstituted to 100 µg/mL in PBS/ 0.1% BSA	500 µL	100 ng/mL
Recombinant Human Noggin, reconstituted to 250 µg/mL in PBS/ 0.1% BSA	200 µL	100 ng/mL
A-83-01, reconstituted to 10 mM in DMSO	250 µL	0.5 µM
Rspo1 conditioned medium	50 mL	10% v/v
SB202190, reconstituted to 15 mM in DMSO	150 mL	1 mM
Antibiotic-Antimycotic (100X)	5 mL	1X

2. ROCK inhibitor (Y-27632) (SCM075) at 10 µM final concentration should be added to 1X Complete Medium only during passaging or in the single cell culture of the lung organoids.
3. Mix well, and filter through a 0.2 µm filter.

Thawing Lung Organoids

1. Prior to thawing human lung organoids, thaw sufficient growth-factor reduced (GFR) Matrigel® domes matrix (Corning, 356231) to seed 4 domes at 25 µL per dome. Place thawed Matrigel® domes on ice.

   Note: 1 cryovial will be thawed into 4 x 25 µL domes.
2. Pre-warm a new sterile 24-well plate in the 37 °C incubator.
3. Pre-wet a 15 mL tube with ice-cold Dulbecco's PBS (D-PBS)/10% FBS, aspirate and then add 10 mL of ice-cold DMEM/F12 PLUS medium (SCM162) to the 15 mL tube.
4. Thaw one vial of human lung organoids by submerging 3/5 of the vial into a 37 °C water bath until only a sliver of ice remains. Spray the outside of the vial with 70% ethanol.
5. In a sterile tissue culture hood, quickly and gently transfer the vial contents into the 15 mL conical tube containing 10 mL of ice-cold DMEM/F12 PLUS Medium (Step 3). Use 1 mL of ice-cold DMEM/F12 PLUS to wash the cryovial and add the rinse to the tube.
6. Centrifuge the 15 mL conical tube at 600 × *g* for 5 min (4 °C). Carefully aspirate the media with a pipet. Discard the majority of the supernatant and keep the last 1 mL in the tube. Organoids should appear as an opaque layer at the bottom of the tube.
7. Add 1 mL of Cell Recovery Solution (Corning, 354253) and gently pipette to wash the Matrigel® domes pieces.
8. Incubate on ice for 1 hour, gently shake the 15 mL tube occasionally throughout the 1-hour incubation period.

9. At the end of the incubation, add 10 mL of ice-cold DMEM/F12 PLUS medium.
10. Centrifuge at 600 × *g* for 5 min (4 °C).
11. Discard the majority of the supernatant, leaving only 10 µL at the bottom of the tube. Place the tube on ice for 3 min to allow the remaining media coating the sides of the tube to settle to the bottom. Then carefully discard the remaining supernatant using a P-200 µL pipette tip.
12. Transfer 110 mL of thawed Matrigel® domes to the organoid pellet. Quickly and gently resuspend the organoid pellet by pipetting up and down 5-10 times with a P-200 pipette, being careful to avoid causing air bubbles. Seed 4-wells of the pre-warmed 24-well plate (Step 2) with 25 µL dome per well.
13. Allow the domes to incubate for 15 minutes in a 37 °C humidified incubator with 5% CO_2.
14. Gently add 1 mL of the complete growth medium containing 10 µM ROCK Inhibitor (SCM075) to each well.
15. Add 500 µL of D-PBS to the surrounding wells to minimize evaporation and incubate the plate at 37 °C 5% CO_2.
16. Growth media should be changed every other day; replace each well with 1 mL fresh complete growth media without ROCK Inhibitor (Y-27632).
17. Cells are passaged once the organoids have recovered and have grown in size (Figure 1). This is typically 4-7 days but may take longer during the first thaw as certain organoid lines may recover more slowly.

Passaging Lung Organoids

1. The optimal time for passaging must be determined empirically for each organoid line. To maintain a continuous healthy culture, the typical split ratio for SCC599 is 1:3 every 10 days. Using this split ratio as a guide, empirically determine the optimal split ratio for each line based on the culture density, the growth rate and the color of the media.

   Note: After thawing, the splitting frequency will likely be variable for the first 2-3 passages.
2. Pre-warm a sterile tissue culture-treated 24-well plate in the 37 °C incubator.
3. Pre-wet a 15 mL tube with ice-cold Dulbecco's PBS (D-PBS)/10% FBS.
4. Matrigel® domes should be thawed on ice prior to use. Prepare enough GFR Matrigel® domes for a volume of 25 µL per dome. For a split ratio of 1:3 (for example, from 4 domes at thaw to 12 domes for the first passage); 300 mL will be required. Add 5% overage = 320 mL total volume.
5. Aspirate the medium from the wells.
6. Add 1 mL of TrypLE® Express (Thermo Fisher, 12605-010) to each well that contains the organoid domes. Use a P1000 pipette tip to scrape the well and break up the Matrigel® dome(s). Place the pipette tip perpendicular to the bottom of the well and expel the organoids with a scraping motion. Pipette up and down 10 times in each well to ensure that the Matrigel® domes pieces are broken up. Return the plate to the 37 °C incubator.
7. Incubate the plate in a 37 °C incubator for 10-15 min, bring the plate back to the hood, use a new P1000 pipette tip to pipette the suspension up and down 10 times. Observe the culture under the microscope and stop pipetting when most of the cell suspension are single cells to small clumps of 10 cells. Transfer the cell suspension into the pre-wet 15 mL tube (Step 3). Rinse each well with 1 mL of ice-cold DMEM/F12 PLUS and add the rinse to the 15 mL tube.
8. Top off the tube with ice-cold DMEM/F12 PLUS and centrifuge at 600 × *g* for 5 min (4 °C).
9. Carefully aspirate the media with a pipet, remove as much residual Matrigel® domes as possible without disturbing
   the organoid pellet. Leave only 10 µL at the bottom of the tube. Place the tube on ice for 3 min to allow the remaining media coating the sides of the tube to settle to the bottom. Then carefully discard the remaining supernatant.
10. For the initial 4-wells at thaw, expand to 12-wells after one passage (1:3 split ratio). Resuspend the pellet in 420 µL Matrigel® domes (25 mL dome per well). Quickly and gently resuspend the organoid pellet about 5-10 times with a P-200 tip. Avoid formation of air bubbles.
11. Seed 12 wells of a new pre-warmed 24-well plate (Step 2) with 25 µL dome per well. Transfer the plate to the 37 °C 5% CO_2 incubator for 15 min for the Matrigel® domes to harden.

    Note: Alternatively, organoids may be plated in a pre-warmed 6-well plate. Aliquot five or six 25 mL organoid domes per well of a 6-well plate.

12. Add 1 mL of the complete growth medium containing 10 µM ROCK inhibitor to each well of a 24-well plate or 3 mL media to each well of a 6-well plate.
13. Incubate the plate at 37 °C 5% CO_2.
14. After 24-hour, exchange media with fresh Complete Growth Medium without ROCK Inhibitor. Exchange media every other day until the next passage.

Single Cell Dissociation of Lung Organoids

Below is an example of single cell passaging starting from 16 domes plated in three wells of a 6-well plate. Modify the protocol based on the plate format used.

1. Pre-warm a new sterile tissue culture-treated 96-well plate in the 37 °C incubator.
2. Pre-wet a 50 mL tube with ice-cold Dulbecco's PBS (D-PBS)/10% FBS.
3. Matrigel® domes should be thawed on ice prior to use.
4. Aspirate the medium from the wells containing the organoid domes.
5. Add 1 mL of TrypLE® Express (Thermo Fisher, 12605-010) to each well, use a cell scraper to scrape off the domes, then break up the Matrigel® domes into smaller pieces by using 1 mL pipette tip. Break organoids by placing the pipette tip perpendicular to the bottom of the well and expel organoids with a scraping motion. Pipette up and down 10 times in each well to ensure that the Matrigel® domes pieces are broken up and return to the 37 °C incubator for 15 min incubation.
6. Bring the plate to the hood; use a P1000 pipette tip to pipette the suspension up and down 10 times. Observe the dissociation under the microscope. Most of the cells should be single cells. If not, incubate for an additional 2-3 minutes then pipette up and down. Check the dissociation under the microscope. For most of the human lung organoid lines, single cell digestion is obtained within 15 min treatment.
7. Once single cells are observed, add 1 mL/well of Complete Growth Medium containing 10 µM ROCK inhibitor to resuspend the cells. Combine the cells to the pre-wet 50 mL tube (Step 3). Count the total cell number on a hemocytometer.
8. Top off the 50 mL tube with DMEM/F12 PLUS, centrifuge at 600 × *g* for 5 minutes (4 °C). Carefully aspirate the media by connecting a P-200 pipette tip to the end of an aspirating pipette. Be careful not to aspirate the pellet.
9. Add the appropriate amount of the thawed GFR Matrigel® domes to the organoid pellet at the desired cell concentrations and resuspend 5-10 times. Avoid generating air bubbles during the pipetting.

   Note: Start at 1000, 2000 or 5000 cells per 10 µL domes to determine the desired concentration.
10. Transfer the plate to the 37 °C incubator for 15 min for the Matrigel® domes to harden. Add 200 µL Complete Growth Medium containing 10 µM ROCK inhibitor to each well.
11. Incubate the plate at 37 °C 5% CO_2.
12. After 24-hour, exchange media with fresh Complete growth Medium containing ROCK Inhibitor. Exchange media every other day until the next passage.

    Note: For single cell passage of organoids, it's important that every passage and media change is in Complete Growth Medium containing 10 µM ROCK inhibitor.

Cryopreservation of Lung Organoids

It is important *not* to dissociate the organoids by enzyme treatment at freezing. Manual disruption of the Matrigel® domes with a pipette tip is sufficient to suspend organoid fragments in freezing medium. We recommend freezing 4-6 x 25 µL domes to 1 cryovial. The average organoid density of each dome should be ~90% at the time of freezing. Prepare Mr. Frosty® container(s) and have it ready at −80 °C. The following is an example of harvesting 16-18 domes in 3 wells of a 6-well-plate (5-6 domes/well), scale up accordingly.

1. Remove the complete growth medium from wells in the 6 well plate and replace with 1 mL/well 3dGRO® Organoid Freeze Medium (SCM301) or alternatively, growth medium containing 10% DMSO.
2. Use the cell scraper to scrape off the domes, then break up the Matrigel® domes into smaller pieces by using 1 mL pipette tip. Pipette up and down 20-30 times. Repeat for all the wells.
3. Consolidate the organoid suspension from all the wells, mix gently, then transfer the organoid suspension into appropriately labeled cryogenic vials at 1 mL/cryovial.

   Note: We typically freeze 4-6 x 25 µL domes into 1 cryovial.
4. Place the cryovials into Mr. Frosty® container(s), and transfer to the −80 °C freezer overnight before moving the vials into liquid nitrogen (−135 °C) for long term storage.

Whole Mount Immunocytochemistry of Lung Organoids

Important Notes Before Starting:

The following protocol is meant to serve as a guidance for first time users and is based on organoids cultured in 24-well plates. The protocol may be modified and adapted once users are more familiar with the process.

- We recommend using a pair of scissors that have been sterilized with 70% ethanol or isopropanol to cut the ends of P-1000 tips to enlarge the opening. Modified P-1000 tips are used to transfer fixed organoids without shearing them. Do not use serological pipettes as they are too bulky to handle small volumes and organoids may stick to the side of the pipettes.
- During PBS washes, gravity is used to collect the organoids. Do not use centrifugation as the centrifugal force will result in mis-shapened organoids.
- 4% paraformaldehyde performs the dual function of fixing the organoids and to help partially dissolve the Matrigel® domes and release the organoids. It is important to remove as much of the Matrigel® domes as possible from the organoids. Matrigel® domes may result in increased background autofluorescence. The more confluent the organoids are inside the domes at the time of fixing, the more readily the Matrigel® domes will dissolve.

1. Prepare a 4% paraformaldehyde (PFA) solution by diluting an 8% PFA Solution (Electron Microscopy Sciences, 157-8-100) 1:1 with 1X PBS.
2. Prepare modified P-1000 and P-200 pipette tips by cutting the ends with a sterilized scissor.
3. Aspirate the medium from each well containing an organoid dome. Wash each well twice with 1 mL 1X PBS. Aspirate between PBS washes.
4. Add 1 mL of the 4% PFA solution to each well. Incubate 45-60 minutes at room temperature on a gently rocking or shaking platform. The shaker/rocker will help expedite detaching the Matrigel® domes and the release of the organoids from the Matrigel® domes.

   Note: GFR Matrigel® domes will partially dissolve when fixed in PFA. At the end of the incubation period, you will notice that many (but not all) the domes are dislodged and that some of the organoids (but not all) will have been released from the domes.
5. Using the modified P-1000 pipette tips, collect any released organoids along with the fixative solution and transfer the contents to a 50 mL conical tube. Allow the organoids to settle to the bottom of the conical tube by gravity (~10-15 minutes). DO NOT CENTRIFUGE.
6. In the meantime, add 1 mL 1X PBS per well to the 24-well plate containing the organoid domes. Incubate 10-15 minutes at room temperature. This is done to dilute the PFA in the dome(s).
7. Carefully aspirate the fixative from the conical tube containing the released organoids (from step 5) and leave a small amount of liquid behind. This will ensure that the organoid pellet will not be aspirated off.

8. Using modified P-1000 pipette tips, collect any released organoids along with the PBS solution from each well (from step 6) and transfer the contents to the 50 mL conical tube. Allow the organoids to settle to the bottom of the conical tube by gravity (~10-15 minutes).
9. Repeat steps 5-8 two more times.
10. Add 0.8 mL of 1X PBS into each well that contains residual organoid domes.
11. Carefully aspirate the supernatant from the conical tube containing the released organoids. Leave a small amount of liquid behind. This will ensure that the organoid pellet will not be aspirated off.
12. Add 4.8 mL 1X PBS to the organoid pellet. Swirl the conical tube to resuspend the organoid pellet. Using a modified P-1000 tip, transfer 200 µL of the organoid suspension into each well containing the 0.8 mL volume of residual organoid domes (from step 10).

    Note: Some organoids may stick to the modified P-1000 tip.
13. If staining will not be performed immediately, seal the 24-well plate containing fixed organoids with parafilm and store in the fridge at 2-8°C for up to 1 month.
14. When ready to perform ICC, transfer the 24-well plate containing the fixed organoids to a dissecting microscope.
15. Using modified P-200 tips (from step 2), pipette 1-4 organoids into each well of an 8-well chamber slide. Remove any residual PBS using an unmodified P-200 pipette tip. Avoid accidentally pipetting up the organoids and shearing them through the P-200 tip.
16. Add 0.4 mL Blocking Buffer (5% horse serum + 0.5% Triton® X-100 in 1X PBS) to each well of an 8-well chamber slide containing the fixed organoids. Block at 2-8 °C overnight or at room temperature for 2-4 hours.

    Note: Use the serum from the same species as the host secondary antibody.
17. Using an unmodified P-200 pipet, remove the blocking buffer while tilting the chamber slide. Avoid pipetting the organoids through the p200 tip.
18. Prepare primary antibodies or directly conjugated antibodies (300-500 µL) in Blocking Buffer.
19. Add primary antibodies. Incubate overnight at 2-8 °C on a gently shaking or rotating platform.
20. Next day, wash 3X with 1X PBS for 10-15 minutes each on the shaking/rotating platform.

    Note: Do not use centrifugation. Remove the PBS with a P-200 pipet after each wash while tilting the chamber slide.
21. Prepare secondary antibodies (300-500 µL) in Blocking Buffer.
22. Add secondary antibodies. Incubate overnight at 2-8 °C on a gently shaking or rotating platform.
23. Next day, wash with 1X PBS for 10-15 minutes on the shaking/rotating platform.

    Note: Do not use centrifugation. Remove the PBS with a P-200 pipet after each wash while tilting the chamber slide.
24. Counterstain with DAPI (5 µg/mL in 1X PBS) for 15-20 minutes.
25. Wash 3X with 1X PBS for 10-15 minutes each on the shaking/rotating platform.

    Note: Do not use centrifugation. Remove the PBS with a P-200 pipet after each wash while tilting the chamber slide.
26. Add 300-400 µL of 1X PBS into each well. Samples are now ready to be imaged on a confocal microscope.

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