Data Sheet

3dGRO® Endometrial Cancer Organoids

Organoid Line

SCC593 and SCC595

Pack Size: ≥1500 viable organoids/vial

Store in Liquid Nitrogen.

FOR RESEARCH USE ONLY

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

Product Overview

Endometrial organoids are advanced three-dimensional cell culture systems derived from endometrial tissue, effectively mimicking the architecture and functionality of the uterus. These organoids serve as critical models for studying endometrial cancer, which includes various histological types with distinct biological behaviors and clinical outcomes.

The predominant subtype, endometrioid adenocarcinoma, constitutes approximately 75–80% of endometrial cancer cases and is classified as Type I, often linked to estrogen exposure and diagnosed at earlier stages. In contrast, aggressive variants such as serous papillary carcinoma (10–15%) and clear cell carcinoma (5–10%) are classified as Type II endometrial cancers, frequently diagnosed at advanced stages and associated with poorer outcomes. Serous papillary carcinoma is particularly aggressive, characterized by high-grade features and mutations in the p53 gene, contributing to its malignancy and poor prognosis.

The complexity of endometrial cancer is further illustrated by mixed endometrial carcinoma, which contains multiple cancerous cell types, and uterine carcinosarcoma, which combines carcinoma and sarcoma components. Additionally, undifferentiated carcinoma, another Type II variant, presents unique challenges due to its aggressive nature. Rare variants, such as neuroendocrine tumors and lymphoepithelial-like carcinoma, further contribute to the diversity of endometrial cancers, emphasizing the need for accurate diagnosis and staging.

We offer two lines of endometrial organoids, 274T (SCC593) and 291T (SCC595), both derived from the serous type of endometrial cancer. The 274T organoid line is positive for PTEN, p53, MUC1, and Pan CD44 expression, while the 291T organoid line is positive for PTEN, MUC16 (CA-125), and MUC1. Both lines are negative for estrogen receptor (ER) and progesterone receptor (PR). These organoids enable researchers to investigate the unique properties of these aggressive cancer types, facilitating the exploration of personalized treatment options and enhancing our understanding and management of endometrial diseases.

Table 1.

ID	Age	Sex	Disease Type	Split Ratio	Days Between Passage	Catalogue No.
274T	92	F	Serous Papillary Carcinoma	1:2	6-8 days	SCC593
291T	68	F	Serous Papillary Carcinoma	1:2	6-8 days	SCC595

Materials Provided (Available separately)

3dGRO® Endometrial Cancer Organoids, 274T (SCC593) *or* 291T (SCC595):
One (1) vial containing ≥1500 viable organoids/vial.

Quality Control Testing

- Viability: ≥1500 viable organoids/vial
- Organoid Growth: Pass
- Negative for infectious disease by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination 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.

Materials Required (Not provided)

Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix (CLS356231), TrypLE™ Express Enzyme (1X), no phenol red (Thermo Fisher, 12-604-013), cultureware and media components (see Protocols).

Representative Data

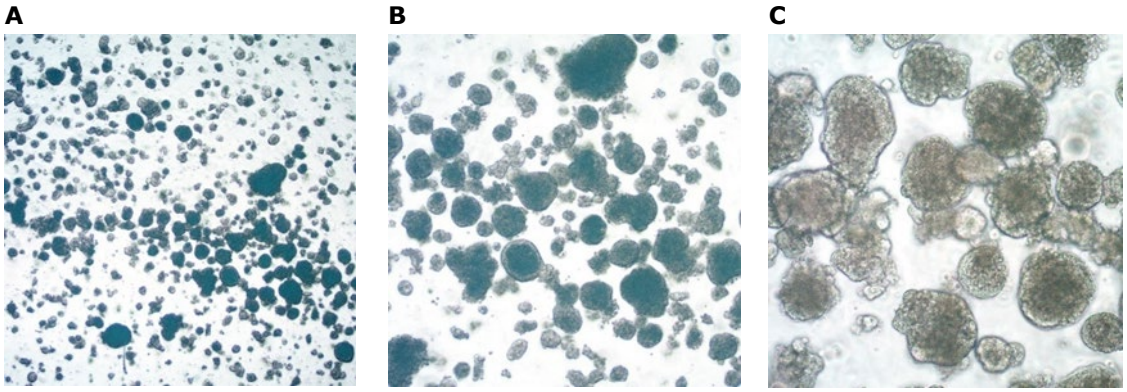

Figure 1. Endometrial cancer organoids on day 4 after thaw at **A.** 2X, **B.** 4X and **C.** 10X magnification.

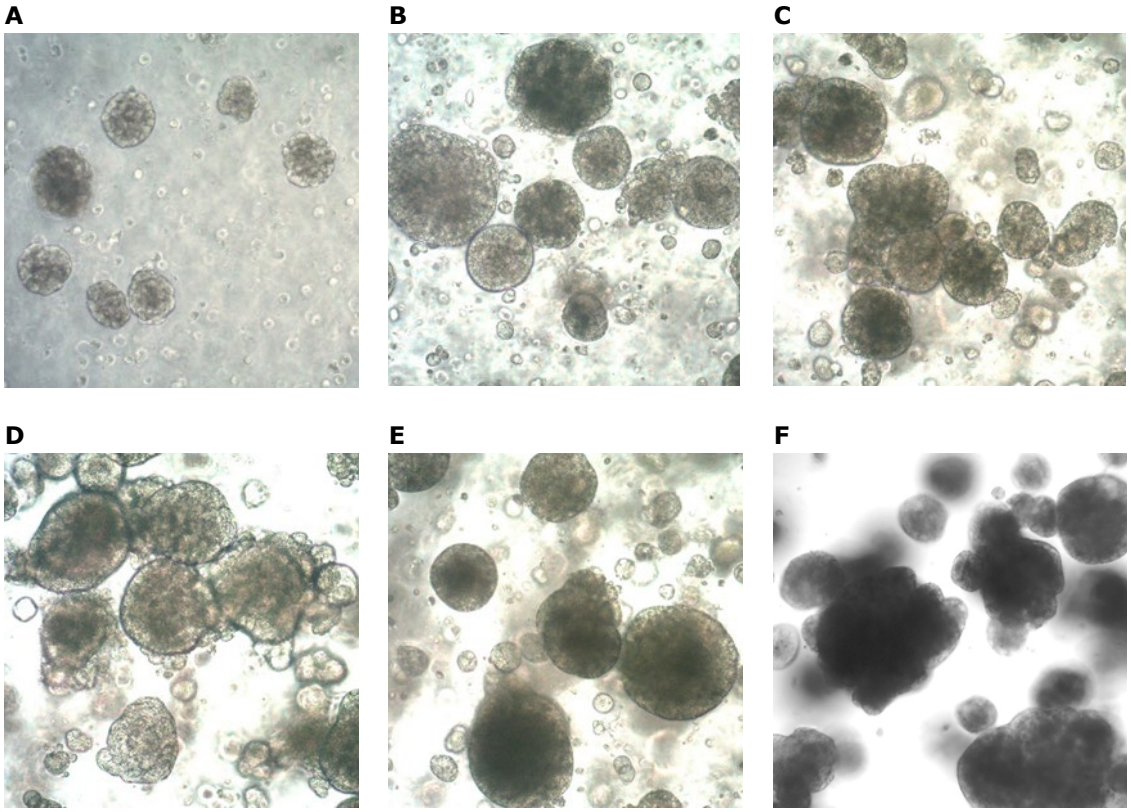

Figure 2. Determining the optimal timing for passaging 3dGRO® Endometrial Cancer Organoids (10X magnification). Representative images of organoids at **A.** day 0, **B.** day 2, C. day 7, **D.** day 8, E. day 9, and **F.** day 13 following passage. Organoids at **E.** day 9 and **F.** day 13 are too dense and are not suitable for passaging. Organoids at day 6 (not shown), **C.** day 7 and **D.** day 8 are suitable for passaging.

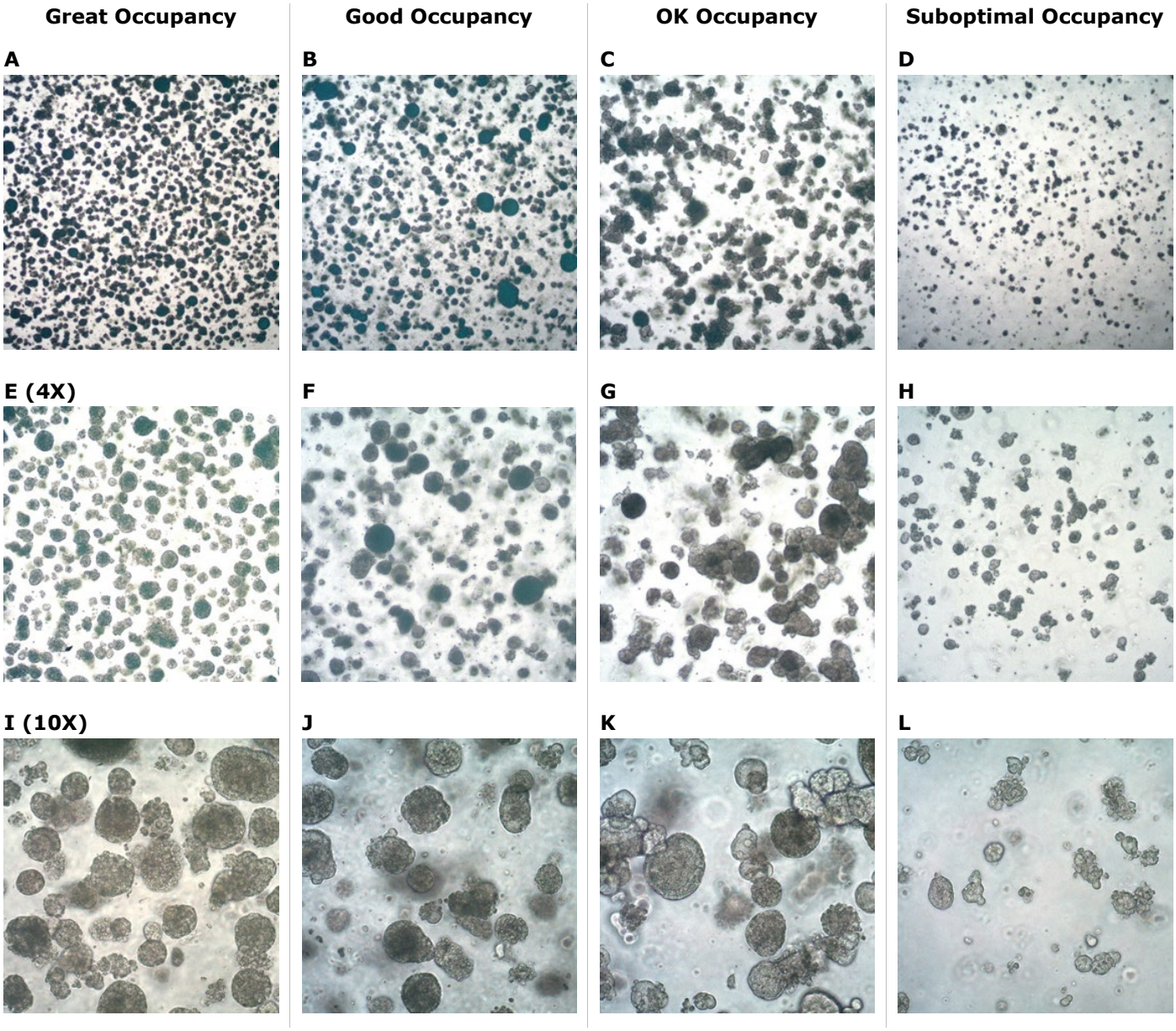

Figure 3. Recommended split ratios for passaging organoids based on percent occupancy. When organoid occupancy is high (**A**, **E**, **I**, "Great occupancy"), a split ratio of 1:3 is advised. For moderate occupancy (**B**, **F**, **J**, "Good occupancy"), passage at a split ratio of 1:2. If occupancy is acceptable (**C**, **G**, **K**, "OK occupancy"), use a split ratio of 1:1.5. In cases of low or suboptimal occupancy (**D**, **H**, **L**), reseeding at a split ratio of 1:1 is recommended.

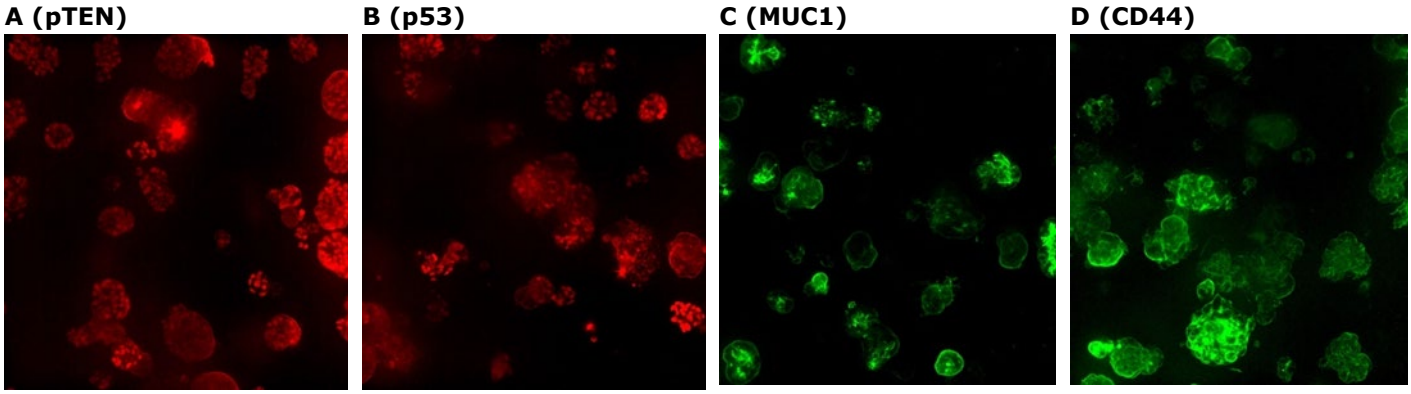

Figure 4. 3dGRO® Endometrial Cancer Organoid 274T (SCC593) expresses **A.** pTEN, **B.** p53, **C.** MUC1, and **D.** CD44.

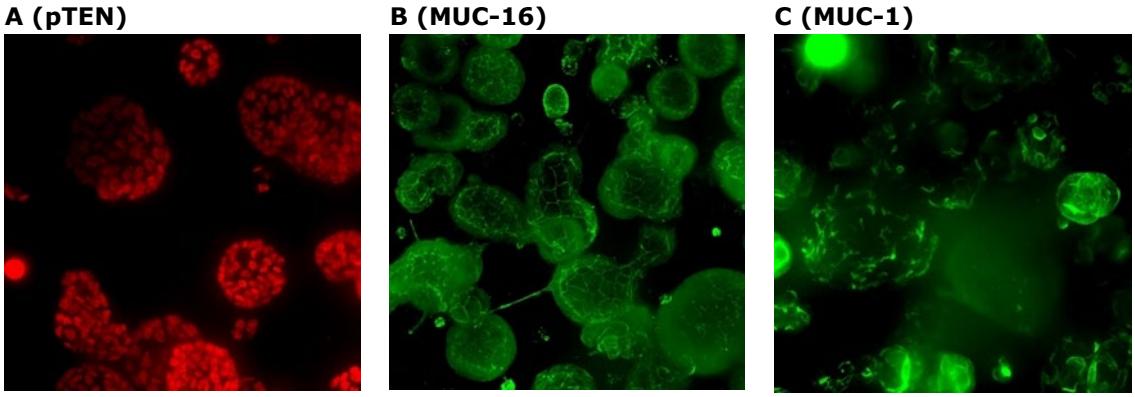

Figure 5. 3dGRO® Endometrial Cancer Organoid 291T (SCC595) expresses pTEN (**A**), MUC-16 (**B**), and MUC-1 (**C**).

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 such as gloves, lab coat, and safety glasses. An example protocol for culturing human endometrial cancer organoids is shown below.

Preparing Complete Endometrial Cancer Organoid Expansion Media (1X)

To make 500 mL Complete Endometrial Organoid Cancer Expansion Medium (1X), combine the following components:

Component	Volume	Final Conc.
DMEM/F12 PLUS Basal Medium	-	1X
3dGRO® R-Spondin1 Conditioned Medium	50 mL	10%
Ala-L-Glutamine Solution (100X), 200 mM	5 mL	2 mM
HEPES Solution, 1M in water	5 mL	10 mM
N-2 Supplement (100X)	5 mL	1X
B27™ Supplement (50X), minus vitamin A	10 mL	1X
N-Acetyl-L-cysteine, prepared as 500 mM solution in water	1.25 mL	1.25 mM
Nicotinamide, 1 M in water	2.5 mL	5 mM
A-83-01, reconstituted to 5 mM in DMSO	20 µL	0.2 µM
SB202190, reconstituted to 20 mM in DMSO	2.5 µL	0.1 µM
β-Estradiol, 100 µM, dissolved in EtOH/DMEM/F12 PLUS Basal Medium	50 µL	10 nM
Human Noggin, reconstituted to 100 µg/mL in PBS/0.1% BSA	0.5 mL	100 ng/mL
Human EGF, reconstituted to 100 µg/mL in PBS/0.1% BSA	0.25 mL	50 ng/mL
Human FGF-2, reconstituted to 100 µg/mL in PBS/0.1% BSA	0.1 mL	20 ng/mL
Human FGF-4, reconstituted to 100 µg/mL in PBS/0.1% BSA	0.25 mL	50 ng/mL
Human HGF, reconstituted to 100 µg/mL in PBS/0.1% BSA	0.1 mL	20 ng/mL
Human IGF-1, reconstituted to 1 mg/mL in PBS/0.1% BSA	20 µL	40 ng/mL
Primocin, 50 mg/mL	1 mL	100 µg/mL
Y-27632 dihydrochloride, 10 mM *	0.5 mL	10 µM

Note: * Y-27632 (ROCK Inhibitor) is added only at the time of thawing or on the first 2 days after passaging. Subsequent media changes do not require ROCK Inhibitor supplementation.

Mix well and filter the 1X Medium through a 0.2 mm filter.

Thawing Organoids

1. Prepare sufficient Growth Factor Reduced (GFR) Matrigel® Matrix (CLS356231; diluted to 8 mg/mL) for six 25 µL domes, plus a 5% overage (total: 157.5 µL). Set aside on ice.
2. Thaw a vial of organoids by submerging 3/5 of the vial into a 37 °C water bath until only a silver of ice remains. Spray the outside of the vial with 70% ethanol or isopropanol.
3. In a sterile tissue culture hood, quickly and gently transfer the vial contents into a 15 mL conical tube containing 8 mL cold DMEM/F12 PLUS and 10 µM Y-27632 (ROCK inhibitor).
4. Centrifuge the 15 mL conical tube for 5 minutes at 700 x *g* at 4 °C.
5. Carefully aspirate the media with a pipet, being cautious to avoid disturbing the organoid pellet. Organoids should appear as an opaque layer at the bottom of the tube. If a clear layer of residual Matrigel® Matrix is visible overlaying the organoid pellet, remove as much as possible without disturbing the organoid pellet.
6. Transfer 157.5 µL of thawed Matrigel® Matrix to the organoid pellet. Quickly and gently resuspend the organoid pellet by pipetting up and down 5–10 times with a P-200 micropipette set at 100 µL, being careful to avoid causing air bubbles.
7. Place organoid suspension on ice for 1 minute to cool the Matrigel® + organoid suspension.
8. Remove the organoid suspension from ice and briefly swirl to mix. Dispense six 25 µL domes into individual wells of a pre-warmed 24-well plate (alternatively, use one well of a 6-well plate). Work quickly and minimize formation of air bubbles during pipetting.
9. Allow the domes to incubate for 10 minutes in a 37 °C humidified incubator with 5% CO_2.
10. Transfer the 24-well or 6-well plate containing the organoid domes to a sterile tissue culture hood. Gently add 1 mL for a 24-well or 3 mL for a 6-well plate of 1X Complete Organoid Medium containing 10 µM ROCK inhibitor into each well containing the organoid domes.
11. Incubate the plate in a 37 °C humidified incubator with 5% CO_2.
12. Monitor organoid growth for 2–4 days after thawing to assess recovery by observation under bright field microscopy. Refer to the website for reference images.
13. Exchange media every 2–3 days with 1X Organoid Media (No ROCK Inhibitor added). Passage 4–5 days after thaw. Passage ratio should be kept at a conservative 1:1.25 to 1:2 for the first 1–2 passages. Once organoids have been established, they may be passaged using the optimal split ratio recommended in Table 1.

    Note: ROCK inhibitor is added only at the time of thawing and on the first 2 days after passaging. Subsequent media changes do not require ROCK inhibitor supplementation.

Passaging Organoids

Prepare enough GFR Matrigel® Matrix for a volume of 25 µL per dome. To maintain approximate equal density, split organoids according to the ratio recommended in Table 1. For example, for a 1:2 split ratio, passage 6 domes into 12 domes.

Optional: Pre-warm a 6-well plate in the 37 °C incubator.

1. Carefully aspirate the media from each well without disturbing the domes. Add PBS to wash the well and carefully aspirate.
2. Add 1 mL TrypLE™ Express plus 10 µM ROCK inhibitor to each well of the 24-well plate or 3 mL TrypLE™ Express plus 10 µM ROCK inhibitor to each well of a 6-well plate.
3. Detach the Matrigel® domes in each well using a P-1000 micropipette. Break the organoids by placing the pipette tip perpendicular to the bottom of the well and expel the organoids with a scraping motion. Repeat multiple times.
4. Incubate the plate for 5 minutes at 37 °C and use a P-1000 micropipette to resuspend up and down 3–6 times to break the organoids. Observe under the microscope and stop when the majority of the cell suspension is comprised of small clumps of 2–3 cells. If the suspension still contains large clumps of cells, repeat the incubation at 37 °C and resuspend again.
5. Once the suspension consists entirely of single cells and small clumps of 2–3 cells, transfer the cell suspension to a conical tube and add equal volume of cold DMEM/F12 PLUS medium. Centrifuge the conical tube for 5–10 minutes at 700 x *g* at 4 °C.

6. Carefully aspirate the media, being careful not to disturb the organoid cell pellet. If a clear layer of Matrigel® Matrix is present overlaying the organoid cell pellet, remove as much Matrigel® Matrix as possible. If the Matrigel® layer is not clear but appears opaque, do not attempt to remove. An opaque Matrigel® layer indicates that the smaller sized organoids that are difficult to pellet may be present in the layer.
7. Transfer the appropriate volume of thawed Matrigel® Matrix to the organoid cell pellet with a P-1000 micropipette. Quickly and gently resuspend the organoid cell pellet about 5 times with a P-1000 tip set to 50 µL below the volume transferred. Avoid causing formation of air bubbles.
8. Dispense 25 µL domes into wells of the culture plate. After each 1 minute of dispensing, place suspension on ice for 1 minute to cool and prevent solidification of the Matrigel® Matrix.
9. Allow the domes to incubate for 10 minutes in a 37 °C humidified incubator with 5% CO_2.
10. Remove the plate from incubator and add 1X Organoid Media containing 10 µM ROCK inhibitor. Exchange media every 2–3 days with fresh media without ROCK inhibitor.

    Note: ROCK inhibitor is added only at the time of thawing and on the day of passaging. Subsequent media changes do not require ROCK inhibitor supplementation.
11. **For applications that require single cell dissociated organoids:** Follow steps 1-11 except that the 1X Organoid Medium should contain 10 mM ROCK inhibitor for the entire duration of the culture.

Cryopreservation of Organoids

Important Notes Before Starting

- We recommend freezing between 3000–6000 organoids per vial.
- Do not dissociate the organoids prior to freezing, either by enzymatic or by mechanical methods. Organoids should be cryopreserved as whole organoids. Do not use enzymatic dissociation to detach organoids during the banking process. Minimize disruption of Matrigel® Matrix by using a pipette tip only enough to allow the organoid pieces to be suspended in freezing medium. Organoids should be frozen a few days before the usual passage day; for example, if passage typically occurs on day 7, then freezing should occur on day 5.
- Patient derived organoids may be frozen in 1X Organoid Media + 10% DMSO.
- Prepare a Mr. Frosty® container and have it ready for storing organoids for freezing down at –80 °C.

1. Remove the plate or dish containing the organoid domes from the 37 °C incubator and place in a tissue culture hood. Aspirate the media.
2. Add sufficient volume of PBS + 10 µM ROCK inhibitor to each well containing the organoids.
3. Carefully aspirate all of the PBS by gradually guiding the P-200 pipet tip connected to the end of an aspirating pipet.
4. Add cold Corning® Cell Recovery Solution at a ratio of 1:10 (for every 1-part Matrigel® Matrix, add 10 parts Cell Recovery Solution). For example, if you have 10 domes of 25 µL each (total 250 µL Matrigel® Matrix), add 2.5 mL
   of Cell Recovery Solution.

   Note: Corning® Cell Recovery Solution is used to depolymerize the Matrigel® Matrix and help recover the organoids in a more efficient manner.
5. Scrape off the Matrigel® domes with a cell scraper.
6. Use a P-1000 pipette set at 800 µL to gently dislodge the Matrigel® domes containing the organoids.

   Important: Keep organoids as intact as possible. Use the expulsion of cold Cell Recovery Solution form the P-1000 pipette to dislodge the Matrigel® domes.
7. Gently pipette and transfer the organoid domes to a 15 mL conical tube. Minimize disruption or breaking of the organoids. Use multiple tubes as needed.
8. Add fresh Cell Recovery Solution to the dish or plate (2–3 mL) to recover the remaining Matrigel® domes. Transfer to the 15 mL conical tube.
9. Place the tube containing the organoids on ice for 30 minutes. Invert the tube 4–5 times during the incubation (every 5–10 min) to facilitate the digestion of Matrigel® Matrix.
10. Centrifuge at 700 x *g* at 4 °C for 5 minutes.

11. Carefully aspirate the media, avoiding disruption of the organoid pellet. If a clear layer of Matrigel® Matrix is present overlaying the organoid cell pellet, remove as much Matrigel® Matrix as possible, If the Matrigel® layer is not clear but appears opaque, do not attempt to remove. An opaque Matrigel® layer indicates that smaller sized organoids that are difficult to pellet may be present in the layer. The residual Matrigel® Matrix should not affect cryopreservation.
12. Resuspend the organoid pellet with freeze medium comprised of 1X Organoid Media containing 10% DMSO.
13. Quickly transfer 1 mL into a cryovial and repeat until all suspension are transferred into cryovials.
14. Quickly transfer cryovials to a Mr. Frosty® container and place in a –80 °C freezer for 24 hours.
15. Transfer the cryovials to liquid nitrogen (–135 °C) for long term storage.

Whole Mount Immunocytochemistry

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. Alternatively, a wide-bore pipette tips can be used.
- During PBS washes, gravity is used to collect the organoids. Do not use centrifugation as the centrifugal force will result in mis-shaped organoids.
- 4% paraformaldehyde performs the dual function of fixing the organoids and to help partially dissolve the Matrigel® Matrix and release the organoids. It is important to remove as much of the Matrigel® Matrix as possible from
the organoids. Matrigel® Matrix 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® Matrix 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. Alternatively, wide-bore pipette tips may be used.
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® Matrix.

   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 or a wide-bore pipette tip, collect any released organoids along with the fixative solution and transfer the contents to a 50 mL conical tube.
6. Add 5 mL of 1X PBS to the 50 mL conical tube to dilute the fixative and allow the organoids to settle to the bottom of the conical tube by gravity (~10-15 minutes). DO NOT CENTRIFUGE.
7. 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).
8. 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.
9. Using modified P-1000 pipette tips or a wide-bore 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).
10. Repeat steps 5–8 two more times.
11. Add 0.8 mL of 1X PBS into each well that contains residual organoid domes.

12. 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.
13. 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 or a wide-bore pipette tip, transfer evenly 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. 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) or a wide-bore pipette tips, 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 Perm/Blocking Buffer (1% (v/v) of Triton® X-100 in 1X PBS + 5% donkey serum) to each well of an 8-well chamber slide containing the fixed organoids. Block at 2–8 °C overnight or at room temperature for 1 hour.

    Note: Use the serum from the same species as the host secondary antibody.
17. Using an unmodified P-200 pipet tip, remove the Perm/Blocking Buffer while tilting the chamber slide. Avoid pipetting the organoids through the P-200 pipette tip.
18. Prepare primary antibodies or directly conjugated antibodies (300-500 µL) in Immunofluorescence (IF) Buffer (0.1% BSA + 0.2% Triton® X-100 + 0.05% Tween®-20).
19. Add primary antibodies to the appropriate well and incubate overnight at 2–8 °C or at RT for 2–3 hours on a gently shaking or rotating platform.
20. Wash 3X with IF Buffer for 10–15 minutes each on the shaking/rotating platform.

    Note: Do not use centrifugation. Remove the IF Buffer with a P-200 pipet after each wash while tilting the chamber slide.
21. Prepare secondary antibodies (300–500 µL) in IF Buffer.
22. Add secondary antibodies to the appropriate well and incubate overnight at 2–8 °C or at RT for 2 hours on a gently shaking or rotating platform.
23. Wash 3X with IF Buffer 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.
24. Counterstain with DAPI (5 µg/mL in 1X PBS) for 15–20 minutes.
25. Wash once 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.
26. Add 300–400 mL of 1X PBS into each well. Samples are now ready to be imaged on a confocal microscope.

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HUB ORGANOIDS

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