Data Sheet

3dGRO® Breast Organoids (Donor 6)

SCC551

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 (PDO) are tissue derived, novel *in vitro* 3D cell models that recapitulate some key features of the respective organ, thus representing a more *in vivo*-like alternative to traditional 2D cell cultures. In addition, PDOs offer possible means to reduce animal model use. PDO maybe derived from resections or biopsy of adult tissue and are phenotypically and genetically stable in culture. PDO are an ideal platform for drug screening, disease modeling and tumor immunity studies, and may also be modified by gene editing.

Worldwide, Breast cancer (BC) is the most commonly diagnosed cancer among women. In the United States, BC accounts for 1 in 3 of all new female cancer cases each year. BC consists of at least 21 distinct histological subtypes and four major molecular subtypes. The grade and cell proliferation may also be used to classify BC subtypes. Almost 81% BC are invasive, and > 75% of such invasive cancers are ductal carcinomas, commonly called invasive ductal carcinoma (IDC). It is important to note that the subtypes show overlaps and there maybe differences in clinical approximations and molecular methods to classify BC.

The major molecular subtypes are:

- Luminal A (HR+/HER2-): This subtype expresses hormone receptors (HR) for estrogen, progesterone, or both. This is the most common BC subtype, is less aggressive and responds to hormone therapy.
- Luminal B (HR+/HER2+): This subtype expresses the epidermal growth factor receptor HER2 and may also be highly positive for the cell proliferation marker Ki67. Luminal B subtype usually shows poorer prognosis than Luminal A.
- Triple negative or basal-like (HR-/HER2-): This subtype does not express HR or HER2, and therefore have the poorest outcome since as it is minimally responsive to hormone therapy or HER2-targeted treatment. Triple negative BC is more common in black or premenopausal women and also in patients with BRCA1 mutation.
- HER2-enriched (HR-/HER2+): With recent advances in HER2-targeted therapies (such as cell therapy), this subtype now shows significantly improved prognosis.

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

Cell Line Characteristics

Sex: Female

Age: 68

Organ: Breast (Tumor)

Disease: Infiltrating Ductal Carcinoma (IDC), ER+/PR+/HER2-

History: High blood pressure (Almodipine/Spironolactone), cholesterol (Pravastatin), asthma (Atrovent/Singulair), migraine (Rizatriptan/Topiramate), nerve pain (Gabapentin/Tramadol), depression (Zoloft).

Store in liquid Nitrogen. The PDO can be cultured for at least 5 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

Viability: ≥1500 viable PDO/vial

Organoid Growth across multiple passages: 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

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 Breast PDO Culture Medium

To make 100 mL of breast PDO culture media, combine the following components. All items may be purchased at SigmaAldrich.com unless otherwise noted.

Component	Quantity	Final Conc.
Advanced DMEM/F12 (Ad-DF)	42.94 mL	1X
3dGRO® L-WRN Conditioned Media Supplement (2X)	50 mL	1X
Recombinant Human Heregulin β-1, prepared as 10 µM solution in D-PBS	50 µL	5 nM, 1X
Recombinant human FGF-10, prepared as 40 µg/mL in DPBS-B*	50 µL	20 ng/mL, 1X
Recombinant human EGF, prepared as 5 µg/mL solution in DPBS-B Human recombinant EGF	100 µL	5 ng/mL, 1X
A83-01, prepared as 5 mM in DMSO	10 µL	500 nM, 1X
B-27™ Supplement (50X), serum free	2 mL	1X
N-acetylcysteine, prepared as 500 mM solution in water	250 µL	1.25 nM, 1X
Nicotinamide, prepared as 1 M solution in D-PBS	1 mL	10 mM, 1X
GlutaMAX™ (100X)	1 mL	1X
HEPES (1 M)	1 mL	1X
Penicillin-Streptomycin (100X)	2 mL	1X
Primocin™ (50 mg/mL)	200 µL	100 µg/mL
Hydrocortisone, prepare 250 µg/mL solution in EtOH/Ad-DF†	200 µL	0.5 µg/mL, 1X
β-estradiol, prepare 100 µM solution in EtOH/Ad-DF‡	100 µL	100 nM, 1X
Forskolin, prepared as 10 mM solution in DMSO	100 µL	10 µM, 1X
Y-27632** (freshly add for thaw/passage)		10 µM

* DPBS-B: Dissolve 0.1 g of BSA (Modified Fraction V) per 100 mL of D-PBS. Filter-sterilize through a 0.22 µm filter. Maybe stored as 1 mL aliquots at −20 °C.

†To prepare a 250 µg/mL stock solution (500 X), dissolve 10 mg in 500 µL of 100% EtOH. Add 39.5 mL of Ad-DF. Filter through a 0.22 µm filter. Maybe stored as single use aliquots at −20 °C.

‡ To make a 100 µM stock solution (1,000 X), dissolve 1 mg in 1 mL of 100% EtOH. Add 35.7 mL of Ad-DF and filter through a 0.22 µm filter. Maybe stored as 100 µL aliquots at −20 °C.

** Y-27632 (Y0503) at 10 µM final concentration should be added to the culture medium only during thawing or passaging of the breast PDO.

Thawing Breast PDO

1. Prior to thawing human breast PDO, thaw sufficient growth-factor reduced (GFR) Matrigel® (Corning 356231). Each cryovial will need 100 µL Matrigel® and will be thawed into 3 x 33 µL domes. Place thawed Matrigel® on ice.
2. Pre-warm a new sterile 12-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 6 mL of ice-cold DMEM/F12 PLUS medium (Cat. No. SCM162) to the 15 mL tube.
4. Thaw one vial of human breast PDO by submerging 3/5 of the vial into a 37 °C water bath until only a little bit 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 6 mL of ice-cold DMEM/F12 PLUS (Step 3).
6. Rinse the vial with 1 mL of ice-cold DMEM/F12 PLUS and add to the tube.
7. Repeat step 6.
8. Centrifuge the 15 mL conical tube at 350 × *g* for 7 min (4 °C). Carefully aspirate the media with a pipette. Discard most of the supernatant and keep the last 1 mL in the tube. PDO should appear as an opaque layer at the bottom of the tube.
9. Add 1 mL of Cell Recovery Solution (Corning 354253) and gently pipette to wash the Matrigel® pieces.
10. Incubate on ice for 1 h. Swirl the conical 2-3 times during the incubation.
11. Add 5 mL ice-cold DMEM/F12 PLUS to the 15 mL tube.
12. Centrifuge the 15 mL conical tube at 350 × *g* for 7 min (4 °C).
13. Discard majority of the sup, leaving only 15-20 µL in the bottom of the 15 mL 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 remove the supernatant with a P-200 pipette tip.
14. Transfer 100 µL of thawed Matrigel® to the 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 3 wells of the pre-warmed 12-well plate (Step 2) with 33 µL dome per well.
15. Allow the domes to incubate for 20 minutes in a 37 °C humidified incubator with 5% CO_2.
16. Add 1 mL of the 1X breast culture PDO media containing 10 µM ROCK Inhibitor (SCM075) to each well. Incubate the plate at 37 °C, 5% CO_2 incubator.
17. Add 500 µL of D-PBS to the surrounding wells to minimize evaporation and incubate the plate at 37 °C 5% CO_2. Figure 1 shows the post-thaw breast PDO.
18. Growth media should be changed every 2-4 days; replace each well with 1 mL 1X breast culture PDO media without ROCK Inhibitor (Y-27632).
19. Cells are passaged once the PDO have recovered and have grown denser. This is typically 10-14 days. However, it might take longer during the first thaw as certain organoid lines may recover more slowly. It is expected that the breast PDO lines will not show robust growth right after thaw.

Passaging Breast PDO

Breast PDO lines are patient specific. Therefore, the optimal passaging time must be determined empirically for each PDO line. For example, to maintain a continuous healthy culture for the NTA line, the typical split ratio is 1:2 every 10-14 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. PDOs secrete factors that stimulate cell proliferation. Therefore, culturing with a PDO density that is not too sparse is a critical factor for a viable culture. The following protocols are standardized for 1:2 split ratio. Volumes should be adjusted for other split ratios.

Note: after thawing, the splitting frequency will likely be variable for the first 2-3 passages.

A. For first passage (3 X 33 µL Matrigel® domes at thaw to 30 X 10 µL Matrigel® domes)

1. Prior to passaging, pre-warm 6-well plates in the 37 °C incubator. Pre-wet 15 mL tube with ice-cold Dulbecco's PBS (D-PBS)/10% FBS. Remove the D-PBS/10% FBS and add 10 mL DMEM/F12 PLUS to the tubes. Keep on ice.
2. Matrigel® should be thawed on ice prior to use. Prepare GFR Matrigel® for a volume of 10 µL per dome. For a split ratio of 1:2 from 3 X 33 µL domes at thaw to 20 X 10 µL domes for the first passage; 200 µL Matrigel® is required. Add 5% overage = 210 µL total volume.
3. Holding the TC plate at a 45° angle and placing a P1000 tip to the side of the well about to be passaged, remove media leaving approximately 250 µL in the well. Do not get close to the Matrigel® dome.
4. Add TrypLE® (ThermoFisher, Cat. no. 12604013) to the well (to a final volume of 1 mL). Dissociate the Matrigel® dome by forcefully pipetting up and down 6 times with a P1000 pipette, aiming at the Matrigel® domes.
5. Repeat step 4 with other wells of the same PDO line by transferring the 1 mL of Matrigel® suspension to the next well and repeating the pipetting process. Up to 3 PDO wells can be combined in 1 mL of TrypLE®.
6. Hold the plate at a 45° angle and forcefully pipette the Matrigel® suspension up and down 10 times. This will dissociate most of the Matrigel® domes, but the PDO may still be intact.
7. To shear PDOs, hold the plate at a 45° angle and forcefully pipette up and down 10 -15 times, firmly pressing the P1000 tip to the bottom corner of the well to create a small opening for PDO disruption. Over shearing the PDOs will result in single cell suspensions, resulting in sparse, slow growing PDO.
8. Transfer the PDO fragments into the 15 mL tube (step 1). Rinse the well once with 1 mL of DMEM/F12 PLUS and pool into the same tube. Up to 6 PDO wells can be pooled in one 15 mL tube.
9. Centrifuge the 15 mL tube(s) at 350 × *g* for 8 min (4 °C). Confirm the presence of pellet by eye. Sometimes the pellet may not be visible by eye.
10. Carefully aspirate the supernatant, leaving behind 200 µL supernatant. Do not touch the bottom of the tube.
11. Place the tube on ice for 2-3 min to allow for any remaining supernatant to collect at the bottom of the tube. Carefully remove the supernatant with a P200 pipette.
12. Add Matrigel® (calculated from step 2) to the pellet and resuspend by gently pipetting up and down 5-8 times with a P200 or P1000 pipette. Take care to not introduce bubbles. Incubate on ice for 5 min.
13. Add the Matrigel® suspension on prewarmed 6-well plates (step 1) as 10 µL drops using a P10 or P20 pipette. Wet the pipette tip with ice-cold D-PBS/10% FBS to prevent PDO sticking to the tip surface. Ideally, each well should have 20 domes.
14. Allow the plate to incubate for 5 min at room temperature. Then transfer the plate to a 37 °C, 5% CO_2 incubator for 20-30 min for the Matrigel® to harden.
15. Add 2 mL of breast PDO culture media (containing 10 µM Y-27632, freshly added) to each well. Incubate the plate at 37 °C, 5% CO_2 incubator.
16. Media should be changed every 2-4 days. No need to add Y-27632 to the growth media when changing media. Periodically check organoid density and morphology with an inverted bright-field microscope (4X or 10X objective).

B. For second passage (20 X 10 µL Matrigel® domes to 40 X 10 µL Matrigel® domes) onwards

1. Prior to passaging, pre-warm 6-well plates in the 37 °C incubator. Pre-wet 15 mL tube with ice-cold Dulbecco's PBS (D-PBS)/10% FBS. Remove the D-PBS/10% FBS and add 10 mL DMEM/F12 PLUS to the tubes. Keep on ice.
2. Matrigel® should be thawed on ice prior to use. Prepare GFR Matrigel® for a volume of 10 µL per dome. For a split ratio of 1:2 from 20 X 10 µL domes at thaw to 40 X 10 µL domes for the first passage; 400 µL Matrigel® is required. Add 5% overage = 420 µL total volume.
3. Holding the TC plate at a 45° angle and placing a P1000 tip to the side of the well about to be passaged, remove media leaving approximately 250 µL in the well. Do not get close to the Matrigel® dome.
4. Add approximately 750 µL TrypLE® (ThermoFisher, Cat. no. 12604013) to the well (to a final volume of 1 mL). Dissociate the Matrigel® dome by scraping and simultaneously pipetting up and down 8 times with a P1000 pipette, aiming at the Matrigel® domes.
5. Add 1 mL ice-cold DMEM/F12 PLUS to the well, hold the plate at a 45° angle and forcefully pipette the suspension up and down 10 times.
6. To shear the PDOs, hold the plate at a 45° angle and forcefully pipette up and down 10-15 times, firmly pressing the P1000 tip to the bottom corner of the well to create a small opening. Over shearing the PDOs in step 4 will result in single cell suspensions, resulting in sparse, slow growing PDO.
7. Transfer the PDO fragments into the 15 mL tube (step 1). Contents of one well of a 6 well plate should be added to one 15 mL tube.
8. Rinse the well once with 1 mL of DMEM/F12 PLUS and pool into the same tube. The 15 mL tube with PDO can be stored on ice for 1 h to harvest multiple wells.
9. Centrifuge the 15 mL tube(s) at 350 × *g* for 8 min (4 °C). Confirm the presence of pellet by eye. Sometimes the pellet may not be visible by eye.
10. Carefully aspirate the supernatant, leaving behind 200 µL supernatant. Do not touch the bottom of the tube.
11. Place the tube on ice for 2-3 min to allow for any remaining supernatant to collect at the bottom of the tube. Carefully remove the supernatant with a P200 pipette.
12. Add Matrigel® (calculated from step 2) to the pellet and resuspend by gently pipetting up and down 5-8 times with a P200 or P1000 pipette. Take care to not introduce bubbles. Incubate on ice for 5 min.
13. Add the Matrigel® suspension on prewarmed 6-well plates (step 1) as 10 µL drops using a P10 or P20 pipette. Wet the pipette tip with ice-cold D-PBS/10% FBS to prevent PDO sticking to the tip surface.
14. Allow the plate to incubate for 5 min at room temperature. Then transfer the plate to a 37 °C, 5% CO_2 incubator for 20-30 min for the Matrigel® to harden.
15. Add 2 mL of breast PDO culture media (containing 10 µM Y-27632, freshly added) to each well. Incubate the plate at 37 °C, 5% CO_2 incubator.
16. Media should be changed every 2-4 days. No need to add Y-27632 to the growth media when changing media. Periodically check organoid density and morphology with an inverted bright-field microscope (4X or 10X objective).

Cryopreservation of Breast PDO

It is important not to dissociate the PDO by enzyme treatment at freezing. Matrigel® domes should be gently disrupted using manual pipetting using ice-cold D-PBS. PDO should be frozen down 2 days prior to the normal passage day. For example, if the PDO are passaged on day 10, they should be frozen down on day 8.
We recommend freezing 15-20 x 10 µL to 1 cryovial. It is advisable to calculate the average number of PDO per 10 µL Matrigel® dome prior to freezing by counting the number of PDO in 8-10 randomly selected Matrigel® domes and calculating the average number.

The following is an example of harvesting 15-20 10 µL domes in 1 well of a 6-well-plate, scale up accordingly.

1. Prepare Mr. Frosty® container(s) and have it ready at –80 °C.
2. Remove the 1X Breast PDO Culture Medium from the well in the 6-well plate.
3. Add 4 mL ice cold D-PBS in the well and gently dislodge the Matrigel® domes using a P1000 pipette.
4. Transfer the D-PBS/Matrigel® suspension to a 15 mL tube.

5. Wash the well with 5 mL ice cold D-PBS and pool into the same 15 mL tube. The 15 mL tube with PDO can be stored on ice for up to 1 h.
6. Centrifuge the 15 mL tube at 350 × *g* for 8 min (4 °C). Confirm the presence of pellet by eye.
7. Aspirate the supernatant carefully without touching the bottom of the tube. Leave behind approximately 200 µL supernatant.
8. Add 1 mL ice cold D-PBS and gently pipette up and down 5 times. Add 9 mL ice cold D-PBS and gently pipette up and down 2-3 times.
9. Centrifuge the 15 mL tube at 350 × *g* for 8 min (4 °C). Confirm the presence of pellet by eye.
10. Aspirate the supernatant carefully without touching the bottom of the tube. Leave behind approximately 50 µL supernatant.
11. Place the tube on ice for 2-3 min to allow for any remaining supernatant to collect at the bottom of the tube. Carefully remove the supernatant with a P200 pipette.
12. Add 1 mL 1X Breast PDO Culture Medium + 10% DMSO. Pipette up and down gently 5 times.
13. Aliquot 1 mL per cryovial. Store the cryovials in Mr. Frosty® container(s) at –80 °C overnight before moving the vials into liquid nitrogen (–135 °C) for long term storage.

Presentation

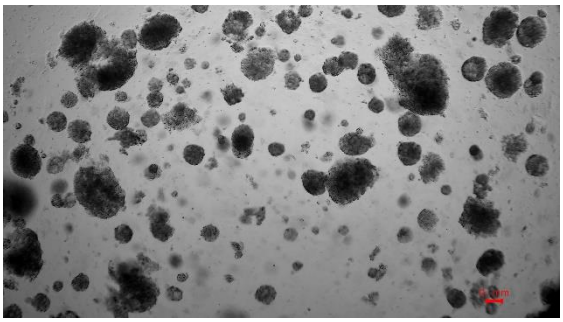

Figure 1: Breast PDO post-thaw.

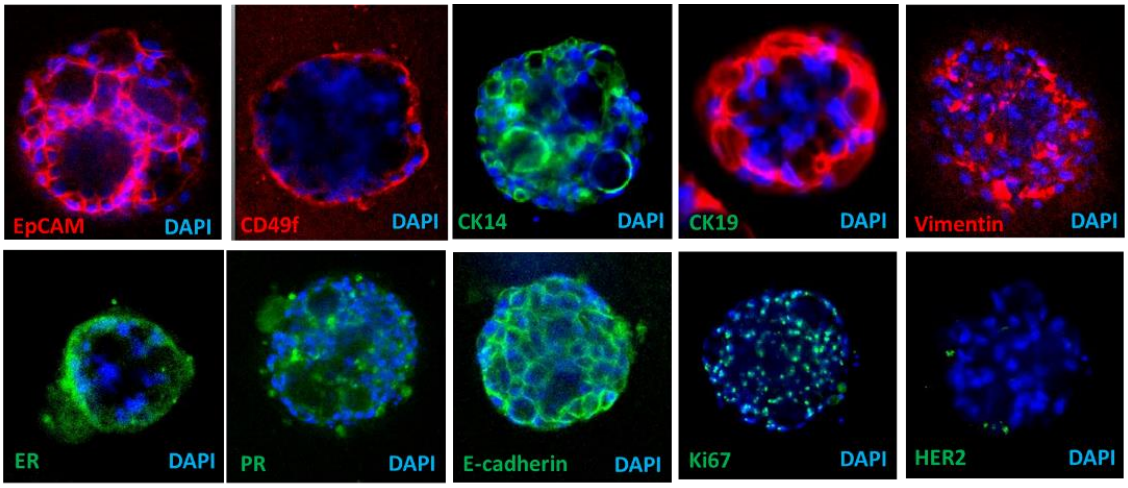

Figure 2: Immunocytochemical (ICC) characterization of breast PDO. Tumor PDO are positive for EpCAM, CD49f, cytokeratin 14, cytokeratin 19, vimentin, ER, PR, E-cadherin and Ki67. PDOs do not express HER2.

For ICC, we recommend growing breast PDO in 10 µL Matrigel® domes in 96-well glass bottom plates and using Visikol® HISTO-M™ Starter Kit (HMSK-1) following the manufacturer's protocol.

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3dGRO® organoids were derived utilizing HUB Organoid Technology.

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