Sigma-Aldrich®

Data Sheet

3dGRO™ Duodenum Intestinal Organoids, Age 55 (Prep 89-D)

Stem Cell Line

Cat. # SCC326 **pack size:** ≥ 1500 organoids/vial

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store at: Liquid Nitrogen

Background

Patient derived organoids (PDOs) are novel in vitro 3D cell models that preserve 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. PDOs can be derived from adult patient biopsies or resected tissues containing native LGR5+ stem cell populations and cultured within an ECM-rich substrate using specialized organoid media such as L-WRN conditioned media. 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.

We are now offering a comprehensive biobank of highly characterized tissue-derived human gastrointestinal organoids from normal and diseased patients. The intestinal organoid biobank contains over 50 highly characterized intestinal organoids from both normal and diseased patients derived from multiple regions of the digestive systems including small intestine (duodenum, ileum), stomach, rectum and colon.

3dGRO™ organoids 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: Male
- Age: 55 Years
- Organ: Duodenum
- Disease: Normal

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.

Quality Control Testing

- Viability: ≥1500 viable 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

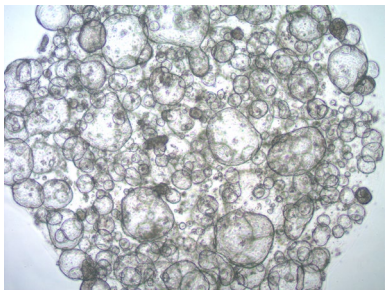

Figure 1. Patient derived organoid morphology. Duodenum Intestinal Organoids, Age 55 (Prep 89-D) after expansion at passage 5.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

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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 Human Intestinal Organoids.

1. To prepare 100 mL of 1X Complete Medium, 50 mL of L-WRN Conditioned Medium (SCM105) is combined with 50 mL 2X Intestinal Media Supplement. The formulation for 2X Intestinal Media Supplement is below.

2X Intestinal Media Supplement (Total volume = 50 mL)

Component	Catalog Number	Volume	2X Conc
DMEM/F12 Plus Basal Medium	Sigma SCM162	42.6 mL	2X
N-2 Supplement (100X)	ThermoFisher 17502048	1 mL	2X
B-27™ Supplement (50X), without vitamin A	ThermoFisher 12587010	2 mL	2X
L-Glutamine Solution (100X), 200 mM	Sigma TMS-002-C	1 mL	2X
HEPES Solution, 1M in water	Sigma H0887	1 mL	20 mM
Niacinamide, prepared as 1M solution in water	Sigma N0636	1 mL	20 mM
N-Acetyl-L-cysteine, prepared as 500 mM solution in water	Sigma A9165	200 µL	2 mM
Human EGF, reconstituted to 200 µg/mL in PBS/ 0.1% BSA	Sigma E9644	50 µL	200 ng/mL
[Leu^{15}]-Gastrin I, reconstituted to 100 µM in PBS/ 0.1% BSA	Sigma G9145	10 µL	20 nM
Prostaglandin E2, reconstituted to 100 µM in DMSO	Sigma P6532	10 µL	20 nM
A-83-01, reconstituted to 1 mM in DMSO	Sigma SML0788	50 µL	1 µM
SB202190, reconstituted to 20 mM in DMSO	Sigma S7067	50 µL	20 µM
Penicillin-Streptomycin, 100x solution	Sigma P4333	1 mL	2X

2. Filter the 2X Intestinal Media Supplement using a 0.2 µm filter.
3. Combine 50 mL of 2X Intestinal Media Supplement with 50 mL of L-WRN Conditioned Medium (SCM105); mix well. Use 1X Complete Media within 2 weeks and store at 2-8°C.
4. Y-27632 (ROCK inhibitor) (Sigma SCM075) at 10 µM final concentration is recommended to be added to 1X Complete Medium with each fresh media change.
5. For increased viability of human gut organoids **during** passaging, calculate the total volume of 1X Complete Medium required for passaging and add 2.5 µM CHIR99021 (Sigma SML1046) to just that volume. CHIR99021 should **only** be present for the first two days of each passage. It is not necessary to add CHIR99021 to the 1X Complete Medium during regular media exchanges.
6. Primocin (Invivogen ant-pm-1) at 100 µg/mL final concentration may be added to 1X Complete Medium as an additional antimicrobial supplement.

Thawing Organoids

1. Prior to thawing human intestinal organoids, thaw sufficient growth-factor reduced (GFR) Matrigel (Corning 356231) for seeding 10 domes of 10 µL per dome. Place thawed Matrigel on ice.
2. Thaw one vial of human intestinal organoids by submerging 3/5 of vial into a 37°C water bath until only a sliver 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 4 mL of 1X Complete Medium and 10 µM ROCK inhibitor.
4. Centrifuge the 15-mL conical tube for 5 minutes at 500*g*.
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 is visible overlaying the organoid pellet, remove as much of the Matrigel as possible without disturbing the organoid pellet.
6. Transfer 100 uL of thawed Matrigel to the organoid pellet. Quickly and gently resuspend the organoid pellet by pipetting up and down 5 times with a P-100 or P-200 micropipette set to 80 µL, being careful to avoid causing air bubbles.

7. Place the organoid suspension on ice for 5 minutes to cool the Matrigel + organoid suspension.
8. Remove the organoid suspension from ice and briefly swirl to mix. Dispense 10-µL domes into one well of the 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 6-well plate containing the organoid domes to a sterile tissue culture hood. Gently add 3 mL of 1X Complete Medium containing 10 µM ROCK Inhibitor and 2.5 µM CHIR99021 into the well containing the organoid domes.
11. Incubate the 6-well plate in a 37°C humidified incubator with 5% CO_2.
12. After 24 h, check for recovery by observation by bright field microscope. Live organoids should begin to show rounded or cystic-like morphology. Replace wells with 3 mL fresh 1X Complete Media containing 10 µM ROCK Inhibitor (without CHIR99021) per well.
13. Exchange media every 1-2 days. By day 4, each dome should contain approximately 100 - 150 organoids.

Passaging Organoids

1. To maintain a continuous culture, human intestinal organoids should be passaged every 5-7 days. Passage of organoids after 7 days is not recommended.
2. Prepare enough GFR Matrigel for a volume of 10 µL per dome. To maintain approximate equal density, split organoids upon passage at least 1:2 (e.g., 10 domes into 20 domes).
3. Prepare ice-cold 1x PBS + 10 µM ROCK inhibitor.
4. Pipette and transfer the medium in the 6-well plate to a 15-mL conical tube, leaving approximately 800 µL behind in the well.
5. Detach the Matrigel domes in the well with a P-1000 micropipette. Break organoids by placing the pipette tip perpendicular to the bottom of the well and expel organoids with a scraping motion. Repeat 10 times.
6. Transfer the organoid suspension to a new 15-mL conical tube.
7. Using the same pipette tip, transfer 1 mL of the existing culture medium from the 15-mL tube (from step 10) and rinse and collect any residual organoid suspensions from the well. Transfer to the conical tube containing the organoid suspension.
8. Centrifuge for 5 minutes at 500*g*.
9. Carefully aspirate the media, being careful not to disturb the organoid pellet. If a clear layer of Matrigel is present overlaying the organoid pellet, remove as much Matrigel as possible. If the Matrigel layer is not clear but appears opaque, do not attempt to remove.
10. Add 1 mL of ice-cold 1x PBS + 10 µM ROCK inhibitor to the organoid pellet.
11. Break the organoids with a P-1000 micropipette by placing pipet tip perpendicular to bottom of tube. Repeat 30 times.
12. Add 4 mL of ice-cold 1x PBS + 10 µM ROCK inhibitor to the organoid suspension.
13. Centrifuge organoid suspension at 4°C for 5 minutes at 500*g*.
14. Carefully aspirate the media with a pipet, removing as much residual Matrigel as possible without disturbing the opaque layer of organoid pellet.
15. Immediately transfer the appropriate volume of thawed GFR Matrigel to the organoid pellet with a P-1000 micropipette. Quickly and gently resuspend the organoid pellet about 5 times with a P-1000 tip set to 20 µL below the volume transferred. Avoid causing formation of air bubbles.
16. Place Matrigel + organoid suspension on ice for 5 minutes.
17. Briefly swirl the organoid suspension to mix, and dispense 10-µL domes into wells of the culture plate. After each 1 minute of dispensing, place suspension on ice for 5 minutes to cool and prevent solidification of the Matrigel.
18. Allow the domes to incubate for 10 minutes in a 37°C humidified incubator with 5% CO_2.
19. Remove plate from incubator and add 1X Complete Medium containing 10 µM ROCK inhibitor and 2.5 µM CHIR99021 in sufficient volume to cover the domes (typically 3 mL per well of a 6-well plate, or 750 µL per well of a 12-well plate).
20. Incubate culture in a 37°C humidified incubator with 5% CO_2.
21. After 24 h, exchange media with fresh 1X Complete Medium containing 10 µM ROCK Inhibitor (without CHIR99021). Exchange media every 1-2 days.

Single Cell Passaging of Organoids

1. Aspirate medium from each well containing an organoid dome.
2. Add 1 mL of 1X PBS to each well. Pipette up and down 5-10 times with a P-1000 pipet to break up the organoid domes and release the organoids. Do this for each well containing the organoid domes.

3. Combine the organoid suspension from all the wells and transfer to a sterile 15 mL conical tube.
4. Centrifuge at 1100 rpm for 5 minutes at 4°C.
5. Carefully aspirate the media by connecting a P-200 pipette tip to the end of an aspirating pipette. Be careful not to aspirate the organoid pellet.

   NOTE: A layer of Matrigel may be visible on top of the organoid pellet. Carefully remove as much of the Matrigel as possible using a P-200 pipette tip.
6. Add 1 mL of TrypLE Express with 10 µM Rock inhibitor to the pellet. Pipette up and down 10 times with a P-1000 pipette tip. Then add another 1-2 mL of TrypLE Express + 10 µM ROCK inhibitor and place in a 37°C incubate at for 30 - 60 minutes. Swirl the tube to mix every 5 minutes. Check a small amount on a hemocytometer at 15 minutes after pipetting up and down a few times to see if organoids are dissociated into single cells. If not, incubate for an additional 10 – 15 minutes with swirling every 5 minutes and check again. Repeat for up to 60 minutes.
7. Once single cells are observed, remove enough sample for counting on a hemocytometer. Then immediately add 5 mL of SCM304 + 10 µM ROCK inhibitor to the rest.
8. Centrifuge at 1100 rpm for 5 minutes at 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. Wash the pellet with an additional 3-5 mL of SCM304 + 10 µM ROCK inhibitor and centrifuge at 1100 rpm for 5 minutes at 4°C.
10. Carefully aspirate the media by connecting a P-200 pipette tip to the end of an aspirating pipette. Be careful to not aspirate the pellet.
11. Quickly add the appropriate amount of the thawed GFR Matrigel+10 µM ROCK inhibitor to the pellet at the desired cell concentration and resuspend 5-10 times. Avoid generating air bubble during pipetting.
12. Immediately place the conical tube containing the cell suspension on ice for 3-5 minutes to cool down. Remove the tube from ice and spray the outside of the tube with 70% ethanol or isopropanol before putting it back into the TC hood.

    NOTE: Start at 5k cells, 10k cells, 15k cells and 20k cells per /25 µL dome to determine the desired concentration.

    TIP: It's important to culture these in SCM304 + 10 µM ROCK inhibitor for up to 8-10 days; otherwise, cells will die.

Cryopreservation of organoids (PDOs)

Important Notes Before Starting:

- We recommend freezing 10 x 10 µL domes to 1 cryovial. The average organoid density of each dome should be ~90% at the time of freezing. If the density is less than 90%, increase the number of domes to freeze.
- PDOs may be frozen down in L-WRN Complete Medium + 10% DMSO or in 3dGRO™ Organoid Freeze Medium (Cat. No. SCM301).
- Prepare Mr. Frosty and have it ready for storing organoid fragments for freezing down at -80°C.

1. Chill enough 1X PBS + 10 µM ROCK inhibitor on ice for at least 30 minutes before starting.
2. Remove the 6-well plate containing 10 µL domes from 37°C incubator and place in a TC hood.
3. Pipette and transfer the medium in the 6-well plate to a 15-mL conical tube, leaving approximately 800 µL behind in the well.
4. Detach the Matrigel domes in the well with a P-1000 micropipette. Break organoids by placing the pipette tip perpendicular to the bottom of the well and expel organoids with a scraping motion. Repeat 10 times.
5. Transfer the organoid suspension to a new 15-mL conical tube. Using the same pipette tip, transfer 1 mL of the existing culture medium from the 15-mL tube (from step 6.15) and rinse and collect any residual organoid suspensions from the well. Transfer to the conical tube containing the organoid suspension.
6. Centrifuge for 5 minutes at 500*g*.
7. Carefully aspirate the media by connecting a P-200 pipette tip to the end of an aspirating pipette. Be careful not to aspirate the organoid pellet. If a clear layer of Matrigel is present overlaying the organoid pellet, remove as much Matrigel as possible. If the Matrigel layer is not clear but appears opaque, do not attempt to remove. This is because there are organoid fragments present in the Matrigel layer. Repeat centrifugation again @ 500*g* for 10 minute.
8. Add 1 mL of ice-cold 1x PBS + 10 M ROCK inhibitor to the organoid pellet.
9. Break the organoids with a P-1000 micropipette by placing pipet tip perpendicular to bottom of tube. Repeat 30 times.
10. Add 4 mL of ice-cold 1x PBS + 10 µM ROCK inhibitor to the organoid suspension.
11. Centrifuge organoid suspension at 4°C for 5 minutes at 500*g*.

12. Carefully aspirate the media by connecting a P-200 tip to the end of an aspirating pipette, removing as much residual Matrigel as possible without disturbing the organoid pellet.
13. Resuspend organoid pellet with freeze medium at 10 µL domes per mL.
14. Quickly transfer 1 mL into a cryovial and repeat until all transferred in cryovials.
15. Quickly transfer cryovials to a Mr. Frosty and place in a -80°C freezer for 24 hrs.
16. 24hrs later, transfer the cryovial to liquid nitrogen (-135°C) for long term storage.

Whole Mount Immunocytochemistry of 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 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 and release the organoids. It is important to remove as much of the Matrigel as possible from the organoids. Matrigel 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 will dissolve.

1. Prepare a 4% paraformaldehyde (PFA) solution by diluting an 8% PFA Solution (Electron Microscopy Sciences Cat. No. 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. 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.
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 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 mL) 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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(subject to local law)

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