Data Sheet

3dGRO® Human Liver Organoid Progenitor Expansion Medium

Stem Cell Medium

SCM313

FOR RESEARCH USE ONLY

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

Product Overview

3dGRO® Human Liver Organoid Progenitor Expansion Medium is a complete ready-to-use serum-free medium for the expansion and long-term culture of human iPSC-derived Liver Organoid Progenitors.

The medium has been validated for use with 3dGRO® Human iPSC-Derived Liver Organoid Progenitors (SCC572) and is expected to also work with patient-derived liver organoids. Liver Organoid Progenitors can be passaged for more than 20 passages in this medium and can support growth of this organoid post thaw from cryopreservation. Organoids propagated in the medium express liver progenitor-specific markers including HNF4a, Sox17, Sox9, and FoxA2.

Materials Provided

Each bottle contains 50 mL of Human Liver Organoid Progenitor Expansion Medium.

Quality Control Testing

- Appearance (Color): Clear/No Particulates (Red Liquid)
- Osmolarity: 350-375 mOsm
- pH: 7.0–7.4
- Sterility Tested: No Growth/Pass
- Endotoxin: <2 EU/mL
- Mycoplasma: Negative
- Functional Assay: Thaw and culture of Human iPSC-derived Liver Progenitor Organoids for 3 passages.

Materials Required (Not supplied)

Reagents

All products are available to order from SigmaAldrich.com unless otherwise noted.

Products	Notes	Supplier	Cat. No.
Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix	Thaw and maintain on ice at all times. Make 1 mL aliquots and store at –20 °C.DO NOT thaw at room temperature or at 37 °C.	Corning	356231
DMEM/F-12 PLUS Basal Media	-	-	SCM162
DMEM-High Glucose	-	-	SLM-241
1 M HEPES Solution	-	-	H0887
Penicillin-Streptomycin (100x)	-	-	P0781
GlutaMAX™ Supplement (100x)	-	Thermo Fisher	35050061
ROCK Inhibitor, Y-27632	Make 10 mM or 1000x stock by reconstituting 5 mg with 1.47 mL sterile water. Aliquot and store long-term at –20 °C.	-	SCM075
24-well tissue culture treated plates	Pre-warmed at 37 °C for at least an hour before plating the organoids. This will help Matrigel® polymerization and spreading of the Matrigel® dome upon plating.	-	M8812
0.25% Trypsin-EDTA solution	Aliquot in 5 mL volume, store at –20 °C. Thaw and pre-warmed to 37 °C before use.	-	T4049
1X D-PBS without Ca^{2+} and Mg^{2+}	-	-	D8537
Fetal Bovine Serum	-	-	ES009-M

Equipment

- Refrigerated centrifuge (e.g., Eppendorf® 5417R, Thermo Fisher Sorvall® ST1 Plus Centrifuge Series, Beckman Coulter Avanti™ J-15R)
- Bright field microscope and camera

Warnings and Precautions

Practice aseptic technique when working with this medium. Do not open the bottle outside biosafety cabinet.

Storage and Stability

See Materials Provided for storage.

Upon receipt, store at –20 °C. When ready to use thaw overnight at 2-8 °C. DO NOT thaw this medium in 37 °C incubator or water bath. Once thawed, mix thoroughly. Use immediately and store at 2-8 °C for up to 2 weeks. Do not re-freeze. Unopened media may be store at –20 °C until the expiry date.

Important Notes Before Starting

The term "domes" refer to organoids that are 3D encapsulated in extracellular matrices such as Matrigel® or collagen.

It is highly recommended that organoids are cultured in medium containing penicillin and streptomycin to prevent bacterial contamination that may be introduced during the long culture process. Users can also substitute penicillin and streptomycin with Primocin® (InvivoGen, ANT-PM-05) for prevention of bacterial, fungal and mycoplasma contamination.

Protocol

Thawing of Stem cell derived Liver Progenitor Organoids (ScLPO) into 24-well plates

1. Prior to thawing of ScLPO, thaw sufficient GFR Matrigel® aliquots for 12 domes at 50 µL per dome + 5% overage (630 µL).

   Note: GFR Matrigel® will polymerize at room temperature; always maintain on ice post thaw.
2. Pre-warm sterile tissue culture-treated 24-well plate in incubator for 1-2 hours prior to thaw. Pre-label the plate with name, date, and passage number of ScLPO.
3. **Prepare Liver Organoid Basal Media:** Add 5 mL penicillin-streptomycin (100X), 5 mL GlutaMAX™ (100 X) and 5 mL of HEPES (1 M) to 500 mL of DMEM/F-12 PLUS Basal media. Store at 4 °C for up to 6 months. The Liver Organoid Basal Media are used for washing, passaging, and thawing ScLPO. Pre-warm media 15 minutes before thawing the ScLPO.
4. Rapidly thaw the frozen vial in a 37 °C bath and transfer contents to the 15 mL tube containing 9 mL of pre-warmed Liver Organoid Basal Media.

   Note: Pipetting at this point should be as gentle as possible as organoids are very fragile at this stage.
5. Add 1 mL prewarmed Liver Organoid Basal Media to wash the cryo-vial and add this to the 15 mL tube.
6. Centrifuge the 15 mL tube at 350 x *g* for 5 min at 25 °C.
7. Carefully discard all the supernatant by aspiration. Remove the remaining supernatant as much as possible with pipettes.
8. Remove the 24-well tissue culture plates from the incubator before resuspending the organoids.
9. Gently resuspend the organoid pellet with 630 µL GFR Matrigel®. Minimize generating air bubbles during pipetting.

   Note: When handling GFR Matrigel®, work as quickly as possible since GFR Matrigel® solidifies at room temperature rapidly.
10. Seed 12 wells of a 24-well plate with 50 µL Matrigel®/organoids dome per well. Let the Matrigel® domes set at room temperature for 2 min, transfer the plate to a 37 °C, 5% CO_2 incubator for 20 min. This will allow sufficient time for the organoid suspension to form a solid dome.
11. Gently add 650 µL of 3dGRO® Human Liver Progenitor Organoid Expansion Medium with into each well containing the organoid domes. To avoid disturbing the domes, dispense the media onto the side of the wells.
12. Incubate ScLPO in a 37 °C, 5% CO_2 humidified incubator overnight.
13. Media should be changed every 3-4 days or whenever the media color turn yellow.

    Note: ScLPO should be passaged when recovered and grown up to the normal passage point. This is typically 8-14 days. The post thaw recovery duration and rate could be longer if ScLPO are stored at –80 °C upon receipts.
14. Each vial has ≥2000 ScLPO, thawed 1 vial to 12 domes of 24-well plate, each dome has ~170 organoids.
15. Observe domes under the microscope and capture images on days 0, 4, 8 and the day of passage for documentation. Morphology and density of ScLPO post thaw at day 0 and day 8 are showed in Figure 1. At day 8, different morphology of ScLPO can be observed as shown in Figure 2.
16. ScLPO may be passaged on day 10-14 after recovery from frozen vial. Following the first passage, the ScLPO should be passaged at 1:8-10 split ratio every 8-10 days using passaging with trypsinization method, or 1:4 split ratio using fragmentation method.

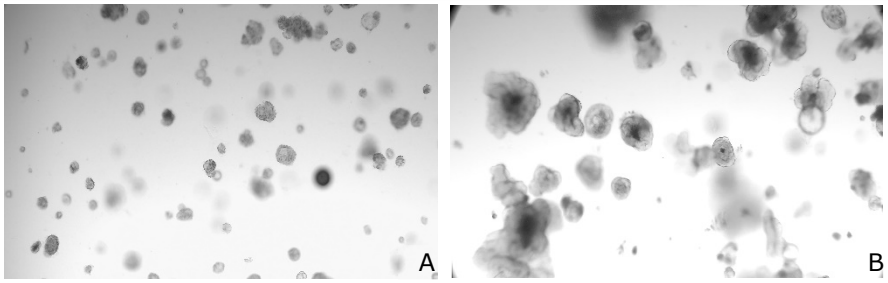

Figure 1: A. Morphology and density of ScLPO at day 0, and **B.** day 8 post thawed using protocol described. Image taken at 4X magnification.

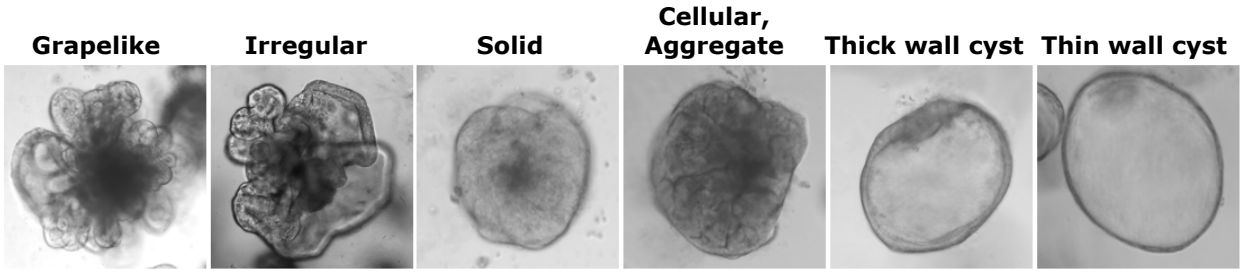

Figure 2: Diversity in ScLPO morphology. ScLPO can appear in different sizes, densities, and structures. Pictures taken at 10X magnification.

Passaging Stem cell-derived Liver Progenitor Organoids using trypsinization methods

Important notes before starting

Make sure the organoids are healthy (with minimum cell death) and are at the exponential grow phase when passaging using this method. Advantage of ScLPO passaged using the trypsinization method:

- each cell or cell cluster give rise to one organoid, thus number of organoids can be estimated during seeding and,
- organoid sizes are homogenous and can be used for biobank and performing functional assays upon differentiation.

1. Pre-warm 24-well plates (1 hour), Liver Organoid Basal Media, DMEM with 10% FBS, 0.25% Trypsin EDTA (15 min) and 3dGRO® Human Liver Progenitor Organoid Expansion Medium at 37 °C.
2. Prior to passaging of ScLPO, thaw enough GFR Matrigel® vials (1 hour before).

   Note: GFR Matrigel® vials should be thawed on ice on the day of use or overnight on ice. GFR Matrigel® vials should be always kept on ice during use.
3. Remove media from the wells. Wash Matrigel® domes with 500 µL DPBS (Ca^{2+} and Mg^{2+} free, room temperature), remove DPBS.
4. Add 450 µL of 0.25% Trypsin EDTA per well and pipette up and down 5-6 times vigorously with P1000 pipette to destroy the Matrigel® dome and release the organoids. This step will help with trypsinization of the organoids.

   Note: Do not digest more than 12 wells of 24-well plate for each digestion batch to avoid over-digestion of organoids by Trypsin. Over-digestion could lead to low viability of ScLPO post passaging.
5. Incubate the plates at 37 °C for 9 mins.
6. Remove plate from 37 °C and pipette up and down 5-6 times each well with P1000 pipette to dissociate organoids into single cells or small cluster of cells. Add 1 mL of DMEM with 10% FBS to each well to neutralize the Trypsin. Collect all the content in each well into a 15 mL tubes (2x 15 mL tubes will be needed if all 12 wells are filled with organoids).
7. Centrifuge at 350 × *g* for 5 min at 4 °C. Remove supernatant, add 10 mL of Liver Organoid Basal Media to wash away the FBS in the DMEM.
8. Centrifuge again at 350 × *g* for 5 min at 4 °C. Remove all the supernatant.

9. Count cells/cell cluster using cell counter. Calculate cell/cell-cluster number required to seed 1500 cells/well.
10. Resuspend gently the calculated amount of Matrigel® and plate 50 µL Matrigel® dome/well of 24-well plates. (Calculation example: To seed 1500 cells/cell clusters per well of one whole 24-well plate, 36,000 cells/cell clusters are need. Resuspend 36,000 cells in 1200 µL of Matrigel® + 5% overage, mix cells and Matrigel® well, plate 50 µL Matrigel® dome/well of 24-well plate).
11. Transfer the plate to a 37 °C, 5% CO_2 incubator for 20 min to polymerize the Matrigel®.
12. Gently add 650 µL of 3dGRO® Human Liver Progenitor Organoid Expansion Medium into each well containing the organoid domes. To avoid disturbing the domes, dispense the media onto the side of the wells.
13. Media should be changed every 3-4 days or whenever the media color turn yellow.
14. Observe domes under the microscope and capture images on Days 0, 4, 8 and the day of passage for documentation. Figure 3 showed morphology of ScLPO cultured in 3dGRO® Human Liver Progenitor Organoid Expansion Medium at different days after passaging using trypsinization method.
15. 3dGRO® Human Liver Progenitor Organoid Expansion Medium should support expansion of ScLPO for >20 passages post thaw.

Figure 3: Morphology of ScLPO passaged using trypsinization method at day 0, 4 and 8.

Passaging Stem cell-derived Liver Progenitor Organoids for maintenance and differentiation (Fragmentation methods)

1. Prewarm 24-well plates (1 hour) at 37 °C, thaw enough vials Matrigel® on ice (1 hour), chill Liver Organoid Basal Media on ice.
2. Remove media from the wells. Add 500 µL of ice-cold Liver Organoid Basal media per well of 24-well plate, scrape with P1000 pipette to dislodge the Matrigel® dome containing organoids and transfer the content of each well to a 15-mL tube. Wash the remaining Matrigel® dome in the well with 100 µL of ice-cold Liver Organoid Basal Media. Top up the 15-mL tube with Basal Media to 10 mL total volume. Chill tube on ice before moving to the next tube/plate.
3. Centrifuge at 350 × *g* for 5 min at 4 °C. Remove supernatant, leaving ~2-2.5 mL of it in the tube.
4. Using a P1000 pipette (set to at least 900 µL volume), pipette up and down vigorously for 10 times to break the large organoids into small fragments or to dislodge small organoids from Matrigel®.
5. Add 8 mL of ice-cold Liver Organoid Basal Media to each tube.
6. Centrifuge again at 350 × *g* for 5 min at 4 °C. Carefully remove all the supernatant including the top part of the transparent pellet containing Matrigel®. This part of the pellet frequently contains death cells.

   Note: It is recommended to attach a P200 pipette tip to the aspirating pipette when performing this step to avoid aspirating the pellet containing the organoids.
7. Resuspend gently with the calculated amount of Matrigel® according to the desire split ratio and number of domes. Plate 50 µL Matrigel® dome/well of 24-well plates.
8. Transfer the plate to a 37 °C, 5% CO_2 incubator for 20 min to polymerize the Matrigel®.
9. Add 650 µL of 3dGRO® Human Liver Progenitor Organoid Expansion Medium per well. Incubate the plate at 37 °C, 5% CO_2 incubator.
10. Change media every 3-4 days or whenever media color turns yellow (this is frequently observed in culture with 80-100% confluency and media can turn yellow within 1-2 days).

11. Split ratio recommendation: 1:4 to 1:5 if the confluency before passage is 80-90%; 1:2 to 1:3 if confluency before passage is 50-70%.
12. Passaging is normally done after 8-10 days of culturing depending on the initial splitting ratio and confluency.
13. Observe domes under the microscope and capture images on Days 0, 4, 8 and the day of passage for documentation. Figure 4 showed the morphology of ScLPO passaged using fragmentation method.

    Note: Smaller and compact organoids (150-250 microns in diameter) might not be efficiently fragmented using this method compared to larger organoids. However, passaging smaller organoids using this method with high splitting ratio allow them to grow to bigger and healthier organoids.

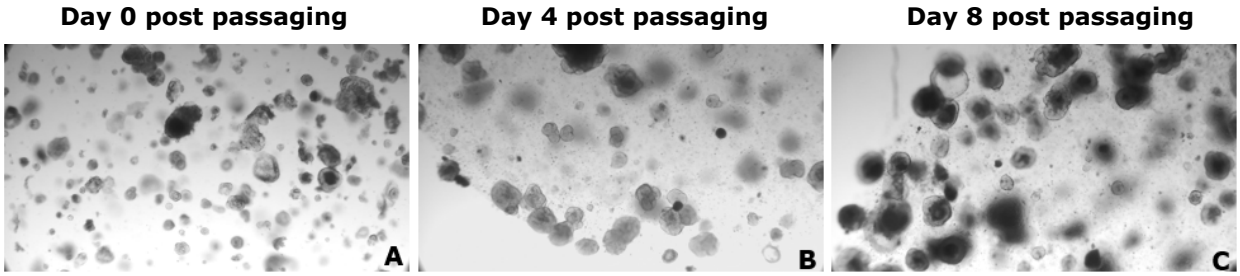

Figure 4: A. Morphology of ScLPO passaged using fragmentation method at day 0, **B.** day 4 and **C.** day 8.

Product Performance

The 3dGRO® Human Liver Progenitor Organoid Expansion Medium should support growth and expansion of ScLPO for at least 3 passages (as high as 20 passages) post thaw. ScLPO cultured in this medium should increase >3 fold in size by 8-10 days of culture (see Figure 3 and 4) using both passaging methods.

Related Products

3dGRO® Human iPSC-derived Liver Organoid Progenitors (SCC572)

References

1. Si-Tayeb et al. (2010). High efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology. 51(1):297-305.
2. Hu et al. (2018). Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell. 175:1591-1606.

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