3dGRO™ Human Colon Organoid Expansion Medium

Stem Cell Media

Cat. # SCM304

pack size: 50 ml

Store at -20°C

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

Data Sheet

Description

3dGRO™ Human Colon Organoid Expansion Medium is a complete ready-to-use serum-free medium for the expansion and long-term culture of human colon organoids.

The medium has been validated for use with 3dGRO™ Human iPSC Derived Colon Organoids (Cat. No. SCC300) and is expected to also work with patient-derived colon organoids. Organoids propagated in the medium express colon-specific markers including the posterior hindgut marker CDX2, α-carbonic anydrase II (CA-II), α-carbonic anhydrase IV (CA-IV), and goblet cell markers Mucin-2 and Mucin-5B.

Storage and Handling

Upon receipt, store at -20°C. When ready to use, thaw overnight at 2-8°C. Once thawed, mix thoroughly. Use immediately and store at 2-8°C for up to 1 week. Do not re-freeze. Unused aliquots may be stored at -20°C until the expiry date.

Quality Control Testing

- Appearance (Color): Clear/No Particlulates (Red Liquid)
- Osmolality: 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 colon organoids for 2 passages.

Representative Images

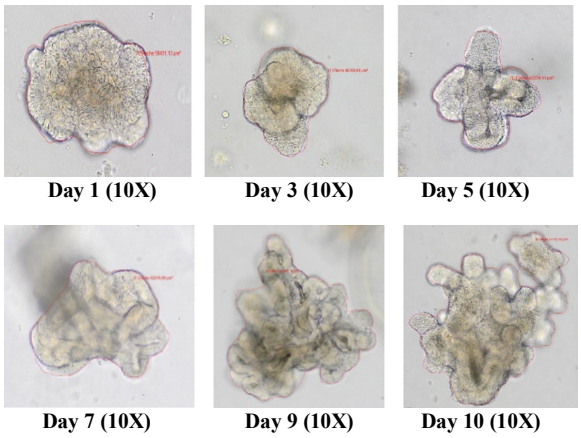

Figure 1. Morphology and growth of human colon organoids. Human colonic organoids were expanded in 3dGRO™ Human Colon Organoid Expansion Medium (SCM304) over a 10-day period and an increase in overall mean length and area was observed.

References

1. Clevers H et al. (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 141(5): 1762-1772.
2. Spence JR et al. (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470 (7332): 105-109.
3. Múnera JO et al. (2017) Differentiation of human pluripotent stem cells into colonic organoids via transient activation of BMP signaling. Cell Stem Cell 21(1): 51-64.
4. Crespo M et al. (2017) Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. Nat Med. 23(7): 878-884.

Ver 1.0/2019-06-13/SCM304/NJA

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Important Notes before Starting:

- The term “domes” refer to organoids that are 3D encapsulated in Growth Factor Reduced (GFR) Matrigel (Corning Cat. No. 356231).
- While not necessary, it is highly recommended that organoids are cultured in medium containing penicillin and streptomycin to prevent contamination that may be introduced during the long culture process.

Reagents Required but Not Provided

Products	Catalog #	Notes
Matrigel® Growth Factor reduced (GFR) Basement Membrane Matrix	Corning 356231	Thaw and maintain on ice. Make 1 mL aliquots. Aliquot the amount needed and maintain on ice. Store unused aliquots at -20°C.
3dGRO™ Human iPSC Derived Colon Organoids or patient derived colon organoids	SCC300	
ROCK Inhibitor, Y-27632	SCM075	Make a 10 mM or 1000X stock by reconstituting 5 mg with 1.47 mL sterile water. Aliquot and store long-term at -20°C.
Penicillin-Streptomycin Solution (100X)	TMS-AB2-C	Aliquot & store long-term at -20°C
24-well tissue culture treated plates	ThermoFisher 142475	
3dGRO™ Organoid Dissociation Reagent	SCM300	
3dGRO™ Organoid Freeze Medium	SCM301	

Thawing Organoids into 24-well plates:

1. Before thawing, prepare sufficient Growth Factor Reduced (GFR) Matrigel for 12 domes at 25 µL per dome + 5% overage (315 µL total). ***Note:*** *GFR Matrigel will gel at room temperature; maintain on ice at all times.*

2. Prepare 3dGRO™ Expansion Medium (Cat. No. SCM304) supplemented with 1X Penicillin/Strep and 10 µM ROCKi. For example, add 250 µL of 100X Pen/Strep solution and 25 µL of 10 mM ROCKi solution (10 µM final) to 25 mL 3dGRO™ Expansion Medium. For media containing ROCKi, prepare fresh on the day of media change.

3. Remove the vial of cryopreserved organoids from liquid nitrogen storage and quickly thaw in a 37°C water bath. Closely monitor until only small ice crystals remain. Quickly remove the vial from the water bath. **IMPORTANT: Do not vortex the vial or leave in the water bath for too long.** Disinfect the outside of the vial with 70% ethanol or isopropanol.

4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the organoid suspension to a sterile 15 mL conical tube containing 9 mL 3dGRO™ Expansion Medium supplemented with 1X Pen/Strep and 10 µM ROCKi (from step 2) Be careful not to introduce any air bubbles during the transfer process.

5. Centrifuge at 1100 rpm for 5 minutes at 4°C. Carefully aspirate the supernatant by connecting a P-200 pipette tip to the end of an aspirating pipette. Be careful not to aspirate the organoid pellet.

6. Immediately transfer 315 µL of the ice-cold GFR Matrigel to the organoid pellet. GFR Matrigel may be viscous; ensure even mixing of the organoid suspension with GFR Matrigel by pipetting up and down several times with a P-1000 pipette. Be careful not to introduce air bubbles during pipetting. Place on ice for 3-5 minutes to cool down the organoid suspension.

   TIP: Set the pipette to 290 µL instead of 315 µL and resuspend the organoid pellet as quickly as you can. This will minimize air bubbles during pipetting.

7. Set a P-200 pipette to 25 µL. Swirl the 15 mL conical tube containing the organoid Matrigel suspension to mix. Dispense 25 µL of the organoid suspension into the center of each well of a 24-well plate. See Figure 3A. **NOTE: Do this as quickly as possible to prevent gelling of the organoid suspension.** Total number of domes = 12. Minimize air bubbles during pipetting.

8. Incubate in a 37°C, 5% CO_2 humidified incubator for 10 minutes. This will allow sufficient time for the organoid suspension to form a solid 3D "dome". See Figure 3A and 2B.

9. Gently add 1 mL of 3dGRO™ Expansion Medium supplemented with 1X Pen/Strep and 10 µM ROCKi into each well containing the organoid domes. To avoid disturbing the domes, dispense the media onto the side of the wells.

10. Incubate in a 37°C, 5% CO_2 humidified incubator overnight.

11. Next day, inspect the organoids with a bright-field microscope. Live organoids should be rounded in shape and not fragmented (dead). Replace with freshly made 3dGRO™ Expansion Medium supplemented with 1X Pen/Strep and 10 µM ROCKi. Incubate at 37°C overnight.

12. On the 2nd day after thaw, replace with fresh 3dGRO™ Expansion Medium supplemented with 1X Pen/Strep and 10 µM ROCKi. Incubate at 37°C overnight. **Note:** ROCKi is only added for the first 2 days after thaw to enhance cell viability. Thereafter, ROCKi is no longer necessary.

13. Replace the media every other day with 3dGRO™ Expansion Medium supplemented with 1X Pen/Strep. **Note.** Media should NOT contain ROCKi.

14. Organoids may be passaged every 10 -12 days of culture using the 3dGRO™ Organoid Dissociation Reagent (Cat No. SCM300). Do not exceed 12 days before passaging.