

3dGRO™ Organoid Freeze Medium

Stem Cell Media

Cat. # SCM301

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

pack size: 50 ml

Store at -20°C



Data Sheet

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Background

Organoids are *in-vitro* derived 3D cell aggregates derived from primary tissue or stem cells that are capable of self-renewal, self-organization and exhibit organ functionality. Organoids address the limitations of existing 2D model systems by providing:

- **Similar composition and architecture to primary tissue:** Organoids harbor small population of self-renewing stem cells (such as intestinal crypt stem cells) that can differentiate into cells of all major cell lineages, with similar frequency as in physiological condition.
- **Relevant models of *in-vivo* conditions:** Organoids are more biologically relevant to any model system and are amenable to manipulate niche components and gene sequence.
- **Stable system for extended cultivation:** Organoids can be cryopreserved as biobanks and expanded indefinitely by leveraging self-renewal, differentiation capability of stem cell and intrinsic ability to self-organize.

Organoids can be cryopreserved using standard cell freezing techniques however freeze/thaw viability remains a challenge. The 3dGRO™ Organoid Freeze Medium is a proprietary optimized cryopreservation media for multiple organoids cell types. The media supports higher freeze/thaw viabilities compared to other commercial or homemade organoid freezing media formulations.

Storage

Aliquot and store the 3dGRO™ Organoid Freeze Medium at -20°C. Thaw at room temperature or 2-8°C overnight. Avoid multiple freeze thaw cycles

Quality Control

Appearance (color): Amber

Osmolality: 250-350 mOsm

pH: 6.8-7.1

Sterility Tested: No Growth/Pass

Bacterial & Fungal Tested: Passed

Cryopreservation Protocol

Freeze ≥ 200 organoids per cryovial when organoids are mature and look like they are ready to be passaged. The following protocol is based on freezing one cryovial.

1. Prepare all media and reagents required before the experiment. Thaw 3dGRO™ Organoid Freeze Medium on ice.
2. Count the number of organoids that are present in a dome. Combine multiple wells if one dome contains less than 200 organoids.
3. Aspirate the culture medium. Add 1 mL PBS to each well. Using a p1000 pipet, pipet up and down 10 times to break up the organoid dome into smaller pieces. Transfer the dissociated organoid mixture, combining domes if necessary, to a 50 mL conical tube.
4. Rinse the wells with 0.5 mL PBS and combine the supernatant to the conical tube. Centrifuge at 500-650x g for five minutes at 4°C,
5. Carefully aspirate the supernatant and leave around 100 µL medium behind. **Note:** Do not aspirate all the way down to the pellet as you may inadvertently aspirate the smaller organoids.
6. Add 10 mL DMEM/F-12 or DMEM medium to wash the pellet. Gently pipette up and down once. Centrifuge the suspension at 650 - 700x g for five minutes at 4°C.
7. Carefully aspirate the supernatant and leave around 50-100 µL medium behind. Using a 20 µL pipette tip, carefully remove the remaining medium.
8. Resuspend the organoid pellet in a suitable amount of ice-cold (2 - 8°C) 3dGRO™ Organoid Freeze Medium. Avoid over-pipetting. Do not dissociate the pellet to single cells. **Note:** Each cryovial should contain at least 200 organoids.
9. Aliquot 1 mL into labeled cryovials. Place the cryovial(s) in a Mr. Frosty container with isopropyl alcohol.
10. Transfer the freezing container to a -80°C freezer; 24hrs later, transfer the cryovial to liquid nitrogen (-135°C) for long term storage. Long-term storage at -80°C is not recommended.

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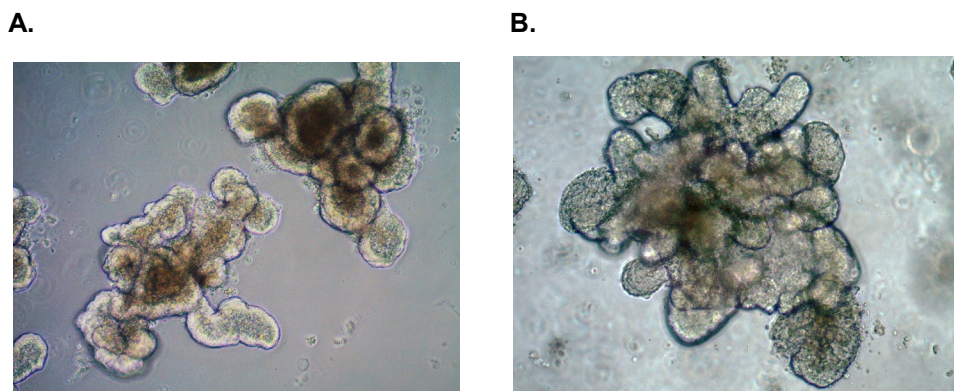


Figure 1. The 3dGRO™ Organoid Freeze Medium supports high freeze/thaw cell viabilities of multiple cell types including mouse intestinal organoids (A) and human iPSC derived colon organoids (B). Images represent day 10 post freeze/thaw in optimized organoid expansion media.

Cryopreservation of Mouse Intestinal Organoids

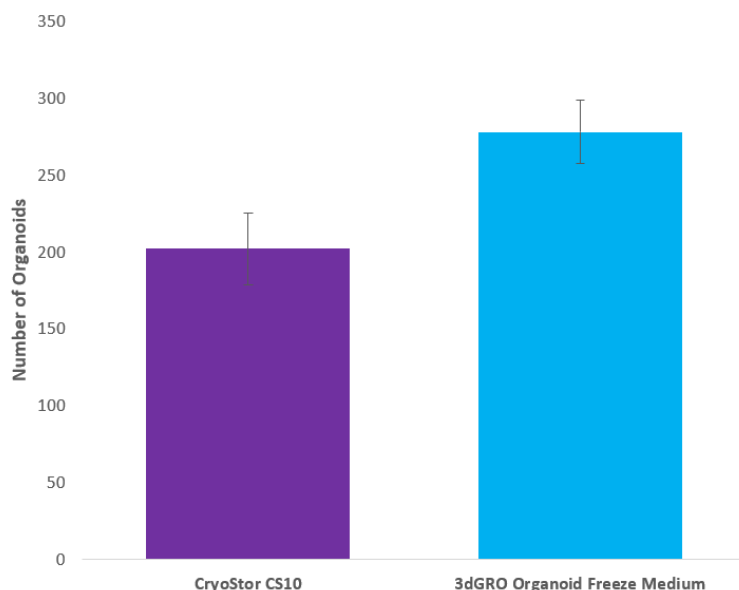


Figure 2. Mouse intestinal organoids cryopreserved in 3dGRO™ Organoid Freeze Medium produce a higher number of viable organoids post freeze/thaw vs. CryoStor CS10.

Related Products

Product Description	Catalog Number
3dGRO™ Human iPSC Derived Colon Organoids	SCC300
3dGRO™ Human Colon Organoid Expansion Medium	SCM304
3dGRO™ R-Spondin-1 Conditioned Media Supplement, 10 mL	SCM104
3dGRO™ Organoid Freeze Medium	SCM301
Definitive Endoderm Induction Medium	SCM302
Hindgut Endoderm Induction Medium	SCM303
DMEM/F-12 PLUS Basal Medium, 500 ml	SCM162

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■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

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