Data Sheet

3dGRO® Organoid Dissociation Reagent

Stem Cell Reagent

SCM300

Pack size: 100mL

Store at room temperature.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

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.

The 3dGRO® Organoid Dissociation Reagent is a proprietary chemically defined enzyme-free dissociation solution used to passage multiple organoids cell types. Due to its chemically defined nature, the reagent produces consistent organoid passaging results.

3dGRO® Organoid Dissociation Reagent can be used in combination with the 3dGRO® R-Spondin-1 Conditioned Media Supplement (SCM104) to isolate and passage primary intestinal organoids from mice or in combination with the 3dGRO® Human iPSC Derived Colon Organoid Expansion Medium (SCM304) to expand human iPSC derived colon organoids (SCC300).

Quality Control Testing

- Appearance: Clear, Liquid
- Osmolality: 340-370 mOsm
- Sterility Tested: No Growth/Pass
- Endotoxin: <2 EU/mL
- pH: 7.2-7.4

Storage and Handling

Store the 3dGRO® Organoid Dissociation Reagent at room temperature. Aliquot into smaller working aliquots before use to avoid reagent contamination issues.

Organoid Passaging Protocol

The following protocol may be used as a general guideline. Different organoid systems may require optimization of the incubation times.

1. Count the number of organoids that are present in a dome. Based on the number of organoids counted, passage such that each new dome(s) will contain at least 50-100 organoids. Thus, for example, if the total number of organoids counted is 100, then plan to passage into 2 new domes.
2. Aspirate the culture medium. Add 1 mL of 3dGRO® Organoid Dissociation Reagent per well of a 24-well plate containing organoid dome(s). Using a p1000 pipet, pipet up and down 10 times to break up the organoid dome into smaller pieces. Transfer the dissociated organoid mixture to a 50 mL conical tube.
3. Rinse each well with 0.5 mL 3dGRO® Organoid Dissociation Reagent. Combine the supernatant to the conical tube.
4. Gently rotate the conical tube for 10 min at room temperature. Add 20-30 mL DMEM/F12 or DMEM to dilute the 3dGRO® Organoid Dissociation Reagent. Centrifuge at 500-650 x *g* for 5 min at 4 °C.
5. Carefully aspirate the supernatant and leave around 100 µL medium.
   Note: Do not aspirate all the way down to the pellet as you may inadvertently aspirate the smaller organoids.
6. Add 10 mL DMEM/F12 or DMEM and centrifuge at 700 x *g* for 5 min at 4 °C.
7. Carefully aspirate the supernatant and leave around 50-100 µL medium. Using a 20 µL pipette tip, carefully remove the remaining supernatant.
8. Based on the number of organoids counted in step 1, resuspend the organoid pellet with a suitable amount of ice-cold growth factor reduced Matrigel® (50 µL/dome).
   Note: Each new dome should contain at least 50-100 organoids.
9. Gently pipet up and down 10-20 times. Avoid generating bubbles. Quickly aliquot 50 µL of the organoid mixture to the center of a well in a new 24-well plate. Avoid bubbles.
10. Incubate the plate at 37 °C for 10 min. Gently add 750 µL of organoid medium to each well; avoid adding directly to the dome. 10 µM ROCK inhibitor should be added to the culture medium for the first 2-3 days after each passage to enhance viability.
11. Change the medium every other day.
12. Passage every 7 days for mouse and 10-12 days for human intestinal and colon organoids. Initially, seed each dome to have 50-100 organoids. The split ratio may be adjusted at later passages based on the organoid system.

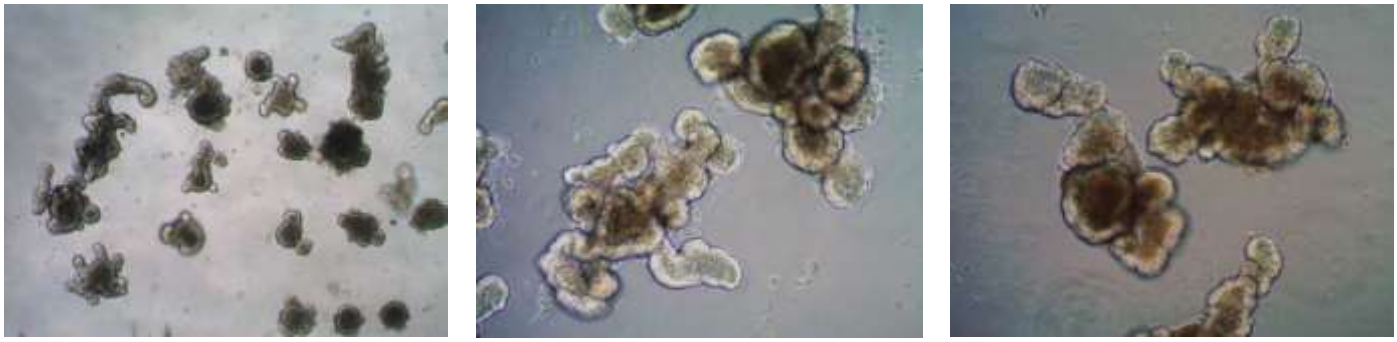

Figure 1. The 3dGRO® Organoid Dissociation Reagent allows for the efficient passaging of primary mouse intestinal organoids at high cell viabilities. Images represent day 10 post passaging in optimized organoid expansion media.

Figure 2. The 3dGRO® Organoid Dissociation Reagent allows for the efficient passaging of human iPS derived colon organoids at high cell viabilities. Images represent day 10 post passaging in optimized organoid expansion media.

Related Products

SCC300; 3dGRO® Human iPSC Derived Colon Organoids

SCM304; 3dGRO® Human Colon Organoid Expansion Medium

SCM104; 3dGRO® R-Spondin-1 Conditioned Media Supplement, 10 mL

SCM301; 3dGRO® Organoid Freeze Medium

SCM302; Definitive Endoderm Induction Medium

SCM303; Hindgut Endoderm Induction Medium

SCM162; DMEM/F-12 PLUS Basal Medium, 500 mL

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