

3dGRO™ R-Spondin-1 Conditioned Media Supplement

Organoid Culture Media

Cat. # SCM104

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

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Background

3D organoid culture systems are increasingly employed as powerful tools for the study of human diseases. R-Spondin proteins are a family of cysteine-rich, thrombospondin type I repeat containing proteins that activate both the WNT/ β -catenin and WNT/PCP pathways. R-spondin-1 (RSPO1) is one of the most extensively used niche factors for culturing 3D organoids and has been used to establish organoids from the stomach, small intestine, colon, pancreas and liver from both mouse and human sources^{1,2,3} along with intestinal organoids from other large and small animals⁴. The 3dGRO™ R-Spondin-1 Conditioned Media Supplement is a potent and inexpensive alternative to purified recombinant RSPO1 protein for organoid cultures and is optimized for the highest level of RSPO-1 expression to ensure consistency and performance.

Source

The 3dGRO™ R-Spondin-1 Conditioned Media Supplement is generated from a 293T cell transfectant stably secreting mouse R-spondin-1 tagged with an influenza hemagglutinin (HA) epitope at the N-terminus and a murine IgG2a Fc fragment at the C-terminus. The conditioned medium does not contain serum.

Storage and Handling

RSPO-1 Conditioned Medium should be stored at -20°C. When ready to use, thaw the supplement overnight at 2-8°C in the dark. Thawed RSPO-1 Conditioned Medium is good for two weeks when stored at 2-8°C.

Quality Control Testing

- Appearance (color): clear, red liquid
- Endotoxin: <2 EU/mL
- Sterility Tested: No Growth/Pass
- Mycoplasma: Negative
- Media are tested for function in supporting growth of human intestinal organoids
- Media are tested for levels of RSPO-1 by ELISA

Representative Data

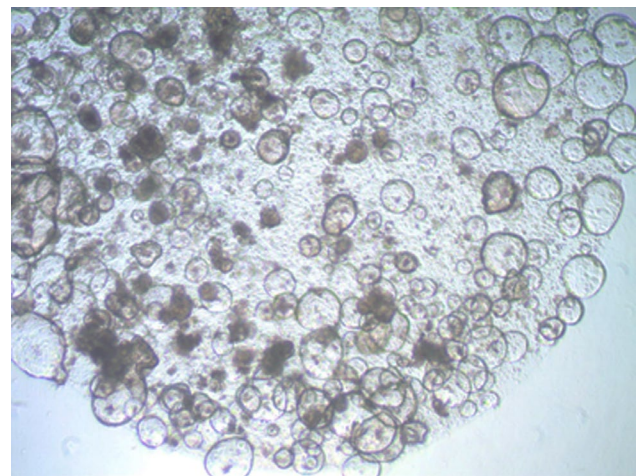


Figure 1. Patient derived 3dGRO™ Colon Intestinal Organoids, Age 21 (SCC321) grown for 5 passages in complete medium containing 50% R-Spondin-1 Conditioned Medium.

References

1. Sato T, Clevers H (2015) *Cell* 161(7): 1700-1700.e1.
2. Ootani A, et al. (2009) *Nat Med* 15(6): 701-706.
3. Sato T, Clevers H (2013) *Science* 340(6137): 1190-1194.
4. Powell RH, Behnke MS (2017) *Biol Open* 6(5): 698-705.

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Protocols

Thawing Medium

1. Thaw medium at 2-8°C overnight, or at room temperature. To minimize loss of growth factor activity, do not thaw medium at 37°C.
2. Once thawed, use immediately and store at 2-8°C for up to two weeks.

Preparing 1X Complete Medium for Human Intestinal Organoids

Note: Below is the optimized formulation for culture and expansion of human intestinal organoids. For non-intestinal organoids that are derived from epithelial tissues, RSPO-1 Conditioned Medium may be used but the 2X Media Supplement for the specific organoid system will have to be optimized by the user.

1. To prepare 100 mL of 1X Complete Medium, 50 mL of R-Spondin-1 Conditioned Medium 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	41.53 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
Wnt3A, reconstituted to 10 µg/mL in water	Sigma GF154	1 mL	200 ng/mL
Noggin, reconstituted to 100 µg/mL in water	PeproTech 250-38	100 µL	200 ng/mL
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 R-Spondin-1 Conditioned Medium; mix well. Use 1X Complete Media within 2 weeks and store at 2-8°C.
4. 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.
5. Y-27632 (ROCK inhibitor) (Sigma SCM075) at 10 µM final concentration is recommended to be added to 1x Complete Medium upon each fresh media change.
6. Primocin (Invivogen ant-pm-1) at 100 µg/mL final concentration may be added to 1x Complete Medium as an additional antimicrobial supplement.

Detailed instructions on the culture and expansion of patient derived human intestinal organoids may be found on the following datasheets (Cat. No. SCC310 – 325).

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

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