User Guide

3dGRO® Wnt3a Conditioned Media Supplement

Catalogue number: SCM112

Pack Size: 50 mL

Store at -20 °C

FOR RESEARCH USE ONLY

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

Background

3D organoid culture systems are increasingly employed as powerful tools for the study of human diseases. Wnt proteins hetero-dimerize Frizzled (Fzd) family receptors and their co-receptors Lrp5/6, triggering downstream signaling pathways including the canonical β-catenin resulting in expression of genes regulating cell development[1]. Wnt3a is considered an essential protein in many organoid culture systems (intestinal, pancreatic, liver, colon etc.) because the proliferation of stem cells is governed by the Wnt signaling pathway[2]. However, due to the high concentration requirement in organoid media, the use of the recombinant Wnt3a becomes prohibitively expensive. Additionally, recombinant Wnt3a proteins are often unstable and lack biological activity. The 3dGRO® Wnt3a Conditioned Media Supplement is a potent and inexpensive alternative to purified recombinant Wnt3a protein for organoid cultures and is optimized for the highest level of Wnt3a expression to ensure consistency and performance.

Source

The 3dGRO® Wnt3a Conditioned Media Supplement is derived from the Mus musculus L Wnt-3A cell line secreting mouse Wnt3a. This product may contain trace amounts of mouse DNA.

Quality Control Testing

- Appearance (color): Clear, red liquid
- Endotoxin: < 2 EU/mL
- Sterility Tested: No Growth/Pass
- Microbial Contamination: Pass
- Mycoplasma: Negative
- Wnt3A biological activity assessed using a Luciferase reporter assay.
- Functional test: Supports culture of human pancreatic organoids ≥ 3 passages.

Storage and Handling

3dGRO® Wnt3a Conditioned Media Supplement should be stored at -20 °C. When ready to use, thaw the supplement overnight at 2-8 °C in the dark. Thawed supplement is good for two weeks when stored at 2-8 °C.

Note: Avoid multiple freeze/thaw cycles.

Representative Data

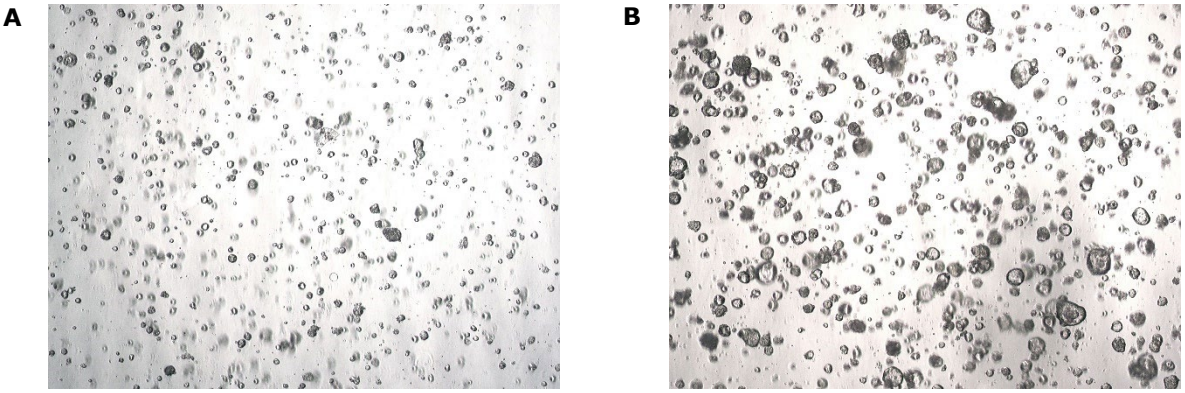

Figure 1. Human pancreatic ductal adenocarcinoma derived organoids (SCC711) grown in complete medium containing 20% 3dGRO® Wnt3a Conditioned Media Supplement (SCM112) plus 30% 3dGRO R-Spondin1 Conditioned Media Supplement (SCM104) at day 4 (**A**) and day 7 (**B**).

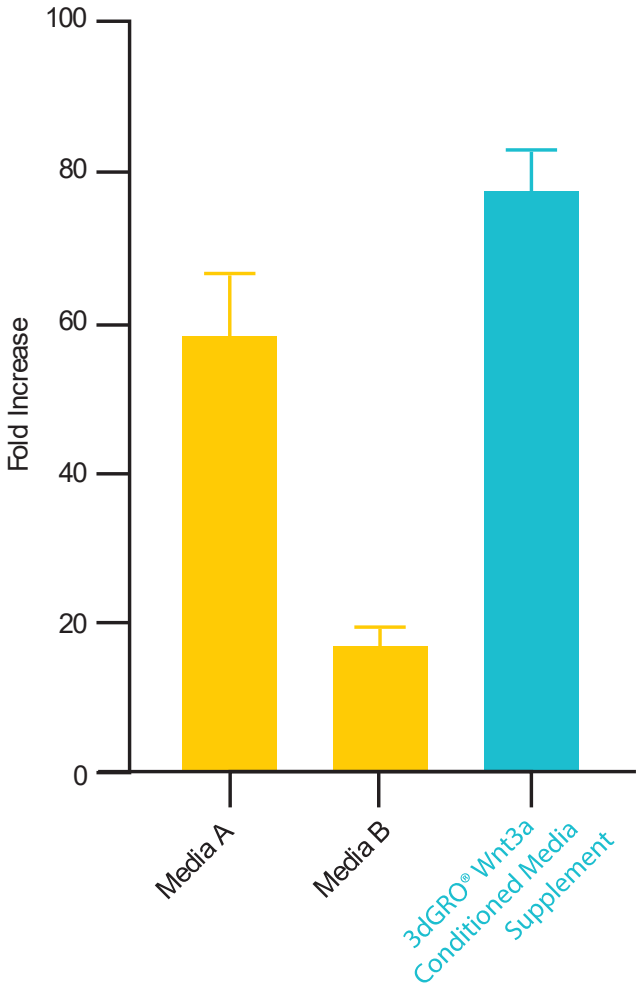

Figure 2. Wnt3a activity level quantified using a luciferase reporter assay. Activity from this product was compared with other Wnt3a conditioned media sources (Media A and Media B). Data expressed as fold increase above untreated control.

Protocols

Thawing Medium

1. Thaw medium at 2-8 °C overnight, or at room temperature in the dark. To minimize loss of growth factor activity, do not thaw medium at 37 °C.
2. Once thawed, use immediately or aliquot and store at -20 °C.

Preparing 1X Complete Medium for Pancreatic Ductal Adenocarcinoma Organoids

The formulation below is recommended for culture and expansion of human pancreatic ductal adenocarcinoma derived organoids. For other tissue-derived organoids, Wnt3A Conditioned Media Supplement may be used but the 2X Media Supplement for the specific organoid system will have to be optimized by the user.

2X Pancreatic Organoid Media Supplement (Total volume = 50 mL)

All items below can be ordered at SigmaAldrich.com unless otherwise noted.

Component	Catalog Number	Volume	Final Concentration in 1X Complete Medium
DMEM/F12 Plus Basal Medium	SCM162	44.39 mL	1X
Ala-Glu (100X), 200 mM	G8541	1 mL	2 mM
B-27™ Supplement (50X)	17504044 (ThermoFisher)	2 mL	1X
N-Acetyl-L-cysteine, prepared as 500 mM solution in water	A9165	250 µL	1.25 mM
HEPES Solution, 1M in water	H3537	1 mL	10 mM
Nicotinamide, prepared as 1M solution in water	N0636	100 µL	1 mM
[Leu^{15}]-Gastrin I, reconstituted to 100 µM in PBS/ 0.1% BSA	G9145	10 µL	10 nM
Recombinant human Noggin, reconstituted to 100 µg/mL in PBS/ 0.1% BSA	GF173	100 µL	100 ng/mL
Recombinant human FGF-10, reconstituted to 100 µg/mL in PBS/ 0.1% BSA	GF172	100 µL	100 ng/mL
A-83-01, reconstituted to 1 mM in DMSO	SML0788	50 µL	0.5 µM
Penicillin-Streptomycin, 100x solution	P4333	1 mL	1X

1. Prepare the 2X Pancreatic Organoid Media Supplement and filter using a 0.2 µm filter.
2. Combine 50 mL of the 2X Pancreatic Organoid Media Supplement with 20 mL of 3dGRO® Wnt3a Conditioned Media Supplement (SCM112) and 30 mL of 3dGRO® R-Spondin1 Conditioned Media Supplement (SCM104). Use 1X Complete Pancreatic Media within 2 weeks and store at 2-8 °C.
3. For the complete culture protocol for expansion and cryopreservation of pancreatic organoids, please consult the specific organoid line product datasheet.

References

1. Merenda A, Fenderico N, Maurice MM. 2020. Wnt Signaling in 3D: Recent Advances in the Applications of Intestinal Organoids. Trends Cell Biol. 30(1):60–73.
2. Sato T, Stange DE, Ferrante M, Vries RGJ, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, et al. 2011. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 141(5):1762–72.

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