

3dGRO™ L-WRN Conditioned Media Supplement

Organoid Culture Medium

Cat. # **SCM105**

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

The 3dGRO™ L-WRN Conditioned Media Supplement is a formulation based on DMEM/F-12 PLUS medium containing the 3 growth factors Wnt3a (**W**), R-spondin-3 (**R**), and Noggin (**N**). These factors act upon the Wnt signaling pathway, which plays a critical role in sustaining the proliferation of LGR5+ crypt stem cells within organoids. Wnt activation is thus essential for in vitro growth of human gut organoids¹. L-WRN conditioned medium is widely used as a component of the culture medium for supporting the growth of human intestinal organoids² including the 3dGRO™ gastrointestinal organoid biobank (SCC310-SCC367). Applications for L-WRN conditioned medium include but are not limited to the expansion of human intestinal gut organoids and other epithelial organoid cell types.

Source

The 3dGRO™ L-WRN Conditioned Media Supplement is derived from the *Mus musculus* L-M(TK-) cell line secreting mouse Wnt3a, R-spondin-3 and Noggin. L-WRN Conditioned Medium may contain trace amounts of mouse DNA.

Storage and Handling

The 3dGRO™ L-WRN Conditioned Media Supplement should be stored at -20°C. When ready to use, thaw the L-WRN Conditioned Medium overnight at 2-8°C in the dark. Thawed medium is good for two weeks when stored at 2-8°C. If media will not be used within two weeks, aliquot unused media and store at -20°C. After thawing refrozen aliquots, do not re-freeze media.

Quality Control Testing

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

Representative Data

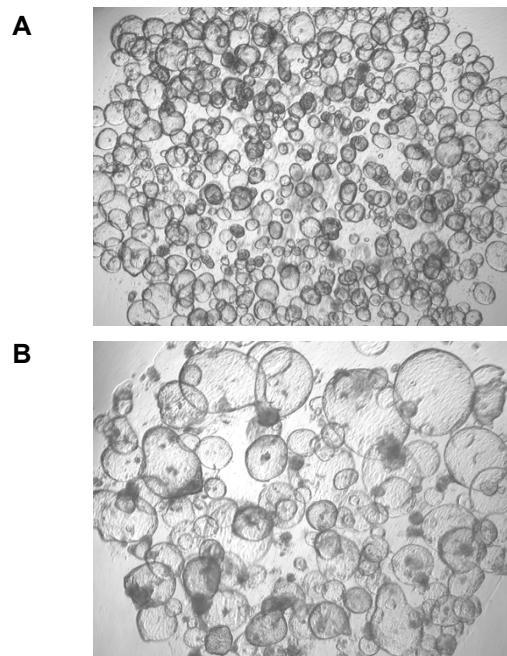


Figure 1. Human colonic organoids grown for 15 passages in complete medium containing 50% L-WRN Conditioned Medium (A). Human duodenal organoids grown for 10 passages in Complete Medium containing 50% L-WRN Conditioned Medium (B).

References

1. *Cell Signal* 2014; 26(3): 570-9.
2. *Gut* 2015; 64(6): 911-920.

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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 or aliquot and store at -20°C.

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, L-WRN 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 L-WRN 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 | 42.6 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 |
| 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 L-WRN 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.

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