



Organoid media formulation #5

Components required

Item	Manufacturer	Catalog #	Storage
Organoid Growth Kit 1E	ATCC	ACS-7104	-20°C or below
L-Glutamine	ATCC	30-2214	-20°C or below
DMSO	ATCC	4-X	2-8°C
Advanced DMEM:F12	Thermo Fisher Scientific	12634028	2-8°C
HEPES	Thermo Fisher Scientific	15630080	2-8°C
B-27 Supplement	Thermo Fisher Scientific	17504-044	-20°C or below
N2-MAX Supplement	R&D Systems	AR009	-20°C or below
HA-R-Spondin1-Fc 293T (RSPO1) Conditioned Media	For each 250 mL of complete media, 50 mL of RSPO1 conditioned media is required. Refer to vendors instructions to prepare conditioned medium from Trevigen Cultrex® HA-R-Spondin1-Fc 293T Cells (Trevigen Cat # 3710-001-01). The protocol for cell culture and conditioned medium generation is available at: https://trevigen.com/docs/protocol/protocol_3710-001-01.pdf		
CRL-2647 L Wnt-3A Conditioned Media	For each 250 mL of complete media, 125 mL of WNT3A conditioned media is required. Refer to the product sheet for instructions to prepare conditioned medium from L Wnt-3A cells (ATCC CRL-2647). The protocol for cell culture and conditioned medium generation is available at: https://www.atcc.org/products/all/CRL-2647.aspx		
Refer to manufacturer documentation for expiration dates and safe handling information.			

Complete 1X growth medium preparation procedure (makes ~250 mL)

1. Thaw B-27, N2-MAX and L-Glutamine on ice or in a refrigerator at 2-8°C. Aliquot stock bottles into working volumes and store at -20°C or below. Avoid multiple freeze/thaw cycles. Thaw DMSO at ambient temperature. Place Organoid Growth Kit at ambient temperature.
2. Prepare supplemented basal medium. Aseptically combine the following components in a sterile 250 mL bottle.

Item	Volume
Advanced DMEM:F12	62.5 mL
HEPES	2.5 mL
L-Glutamine	2.5 mL
B-27	5.0 mL
N2-MAX	2.5 mL
Total volume	75.0 mL

3. Briefly centrifuge the vials in the Organoid Growth Kit to ensure the material is at the bottom of the vial.



- Aseptically reconstitute the individual kit components in the indicated buffer. After adding buffer to each vial, incubate for 15 minutes at room temperature. Mix by repeated pipetting. If the N-Acetyl Cysteine is difficult to dissolve, periodic vortexing and incubation in a 37°C water bath for 10-20 minutes can help the material enter solution.

Item	Catalog #	Buffer	Volume of buffer
Noggin	ACS-7200	Supplemented basal medium	1.0 mL
EGF	ACS-7203	Supplemented basal medium	1.0 mL
FGF-10	ACS-7204	Supplemented basal medium	1.0 mL
SB 202190	ACS-7213	DMSO	0.1 mL
A 83-01	ACS-7209	DMSO	0.1 mL
Nicotinamide	ACS-7214	Supplemented basal medium	2.5 mL
N-Acetyl-Cysteine	ACS-7215	Supplemented basal medium	1.0 mL

Note: Once reconstituted components should be used immediately. Do not store reconstituted components.

- Aseptically combine the reconstituted kit components, conditioned media, and supplemented basal media.

Item	Volume
RSPO1 Conditioned Media	50.0 mL
Wnt-3A Conditioned Media	125.0 mL
Supplemented basal medium	68.5 mL
Noggin	1.0 mL
EGF	1.0 mL
FGF-10	1.0 mL
SB 202190	0.1 mL
A 83-01	0.1 mL
Nicotinamide	2.5 mL
N-Acetyl-Cysteine	1.0 mL
Total volume	~250.0 mL

- Aseptically filter the complete growth medium through an 0.22 µm PES bottle-top filter unit.
- (Optional) Place the supplied sticker on the final collection bottle to indicate media preparation is complete. Label with an expiration date 4 weeks from date of preparation.

Notes

- Once prepared, store complete medium at 2-8°C in the dark.
- Complete medium expires after 4 weeks or at the expiration date of any of the components, whichever comes first.
- Do not freeze complete medium and avoid extended light exposure.



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