Organoid media formulation #6

Components required

Item	Manufacturer	Catalog #	Storage
Organoid Growth Kit 1G	ATCC	ACS-7106	-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
TGF-beta 1	R&D Systems	240-B	-20°C or below
HA-R-Spondin1-Fc 293T (RSPO1) Conditioned Media	For each 250 mL of complete media, 25 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 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 basal medium. Aseptically combine the following components in a sterile 250 mL bottle.

Item	Volume
Advanced DMEM:F12	90.0 mL
HEPES	2.5 mL
L-Glutamine	2.5 mL
B-27	5.0 mL
Total volume	100.0 mL

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

4. 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
EGF	ACS-7202	Supplemented basal medium	1.0 mL
Nicotinamide	ACS-7214	Supplemented basal medium	1.0 mL
N-Acetyl-Cysteine	ACS-7215	Supplemented basal medium	2.5 mL
FGF-10	ACS-7204	Supplemented basal medium	1.0 mL
Gastrin	ACS-7208	Supplemented basal medium	0.5 mL

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

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

Item	Volume
RSPO1 Conditioned Media	25.0 mL
Wnt-3A Conditioned Media	125.0 mL
Supplemented basal medium	94.0 mL
EGF	1.0 mL
Nicotinamide	2.5 mL
N-Acetyl-Cysteine	1.0 mL
FGF-10	1.0 mL
Gastrin	0.5 mL
Total volume	~250.0 mL

6. Aseptically filter the complete growth medium through an 0.22 µM PES bottle-top filter unit.
7. (Optional) Place the sticker supplied with the CoreKit on the final collection bottle to indicate media preparation is complete. Label with an expiration date 4 weeks from date of preparation.
8. Reconstitute the TGF-beta 1 according to the manufacturer's instructions to a final concentration of 20 ug/mL. For example, add 0.5 mL of sterile filtered 4 mM HCl containing 1 mg/mL BSA to a 10 ug vial of TGF-beta 1.
9. For the first 3 weeks of culture only, supplement the complete media prepared above with the TGF-beta 1 to a final concentration of 5 ng/mL by adding 1 µL per 4 mL complete growth medium. After 3 weeks TGF-beta 1 is no longer included in the complete growth medium.

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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