Organoid media formulation #1

Components required

Item	Manufacturer	Catalog #	Storage
Organoid Growth Kit 1A	ATCC	ACS-7100	-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 Sci.	12634028	2-8°C
HEPES	Thermo Fisher Sci.	15630080	2-8°C
B-27 Supplement	Thermo Fisher Sci.	17504-044	-20°C or below
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 supplement basal medium. Aseptically combine the following components in a sterile 250 mL bottle.

Item	Volume
Advanced DMEM:F12	240 mL
HEPES	2.5 mL
L-Glutamine	2.5 mL
B-27	5.0 mL
Total volume	250.0 mL

3. Briefly centrifuge the vials in the Organoid Growth Kit to ensure the contents are at the bottom of the vials.

4. Aseptically reconstitute the components in the indicated buffer. After adding buffer, 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-7202	Supplemented basal medium	1.0 mL
Gastrin	ACS-7208	Supplemented basal medium	0.5 mL
Nicotinamide	ACS-7214	Supplemented basal medium	2.5 mL
N-Acetyl-Cysteine	ACS-7215	Supplemented basal medium	1.0 mL
SB 202190	ACS-7211	DMSO	0.1 mL
A 83-01	ACS-7209	DMSO	0.1 mL

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

5. Aseptically prepare the complete growth medium formulation by combining the reconstituted prepared kit components with the supplemented basal medium.

Item	Volume
Supplemented basal medium	244 mL
Noggin	1.0 mL
EGF	1.0 mL
Gastrin	0.5 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

6. Aseptically filter the complete growth medium through an 0.22 µM PES bottle-top filter unit.
7. (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.