Organoid media formulation #6

The following components are required for media preparation:

Item	Vendor	Catalog #	Storage
Organoid Growth Kit 1G	ATCC	ACS-7106™	-20°C or below
Advanced DMEM:F12	Thermo Fisher	12634028	2-8°C
HEPES	Thermo Fisher	15630080	2-8°C
B-27 Supplement	Thermo Fisher	17504-044	2-8°C
L-Glutamine	ATCC	30-2214™	-20°C or below
Dimethyl sulfoxide (DMSO)	ATCC	4-X™	2-8°C
TGF-beta 1	Bio-techne	240-B-010	-20°C or below
CRL-3797 293T-RSPO1 (RSPO1) Conditioned Media	For each 250 mL of complete media, 25 mL of RSPO1 conditioned media is required. Refer to the product sheet for instructions to prepare conditioned medium from 293T-RSPO1 cells (ATCC CRL-3797). The protocol for cell culture and conditioned medium generation is available at: https://www.atcc.org/products/CRL-3797.aspx		
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/CRL-2647.aspx		

Refer to the manufacturer of individual components for important safety and handling considerations.

ATCC ACS-7106: Organoid Growth Kit 1G Contents

Item	Quantity	Catalog #	Storage
EGF	1 vial	ACS-7202	-20°C or below
FGF-10	1 vial	ACS-7204	-20°C or below
Gastrin	1 vial	ACS-7208	-20°C or below
Nicotinamide	1 vial	ACS-7214	-20°C or below
N-Acetyl Cysteine	1 vial	ACS-7215	-20°C or below
Kit label	1 sticker	ACS-7106-LBL	Ambient

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 the Organoid Growth Kit at ambient temperature.

2. Prepare the supplemented 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. 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
FGF-10	ACS-7204	Supplemented Basal Medium	1.0 mL
Gastrin	ACS-7208	Supplemented Basal Medium	1.0 mL
Nicotinamide	ACS-7214	Supplemented Basal Medium	2.5 mL
N-Acetyl Cysteine	ACS-7215	Supplemented Basal Medium	1.0 mL

4. Aseptically reconstitute TGF-beta 1 according to the manufacturer's instructions to prepare stock concentration. We recommend incubating in buffer for 10-20 minutes at room temperature. For the first three weeks of culture, TGF-beta 1 should be added in complete growth media. After three weeks, TGF-beta 1 is no longer required.

Item	Size	Buffer	Volume of Buffer	Final Concentration
*TGF-beta 1	10 µg	4 mM sterile filtered HCl	1.0 mL	10 µg/mL

* For the first three weeks of culture, TGF-beta 1 should be added in complete growth media to a final concentration of 5 ng/mL by adding 1 µL stock concentration per 2mL complete growth medium. After three weeks, TGF-beta 1 is no longer required.

5. Prepare the complete growth medium formulation:

Item	Volume
Supplemented Basal Medium	95 mL
RSPO1 conditioned media	25 mL
WNT3A conditioned media	125 mL
EGF	1.0 mL
FGF-10	1.0 mL
Gastrin	1.0 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.