



## Organoid media formulation #8

### Components required

Item	Manufacturer	Catalog #	Storage
Organoid Growth Kit 1F	ATCC	ACS-7105	-20°C or below
ROCK Inhibitor Y27632	ATCC	ACS-3030	-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
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: <a href="https://trevigen.com/docs/protocol/protocol_3710-001-01.pdf">https://trevigen.com/docs/protocol/protocol_3710-001-01.pdf</a>		
Refer to manufacturer documentation for expiration dates and safe handling information.			

### ATCC ACS-7105 Organoid Growth Kit 1F Contents

Item	Quantity	Catalog #	Storage
Noggin	1 vial	ACS-7200	-20°C or below
EGF	1 vial	ACS-7203	-20°C or below
FGF-10	1 vial	ACS-7206	-20°C or below
FGF-7	1 vial	ACS-7207	-20°C or below
A 83-01	1 vial	ACS-7209	-20°C or below
SB 202190	1 vial	ACS-7212	-20°C or below
Nicotinamide	1 vial	ACS-7214	-20°C or below
N-Acetyl-Cysteine	1 vial	ACS-7215	-20°C or below
Heregulin	1 vial	ACS-7217	-20°C or below
Kit label	1 sticker	ACS-7105	Ambient

**This formulation requires additional supplementation with ACS-3030 (ROCK Inhibitor Y27632) prior to use. ACS-3030 is not included in the kit. See notes section.**

### **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 supplemented basal medium. Aseptically combine the following components in a sterile 250 mL bottle.

Item	Volume
Advanced DMEM:F12	215.0 mL
HEPES	2.5 mL
L-Glutamine	2.5 mL
B-27	5.0 mL
<b>Total volume</b>	<b>225.0 mL</b>

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
Noggin	ACS-7200	Supplemented basal medium	1.0 mL
EGF	ACS-7203	Supplemented basal medium	1.0 mL
FGF-10	ACS-7206	Supplemented basal medium	1.0 mL
FGF-7	ACS-7207	Supplemented basal medium	1.0 mL
A 83-01	ACS-7209	DMSO	0.1 mL
SB 202190	ACS-7212	DMSO	0.1 mL
Nicotinamide	ACS-7214	Supplemented basal medium	2.5 mL
N-Acetyl-Cysteine	ACS-7215	Supplemented basal medium	1.0 mL
Heregulin	ACS-7217	Supplemented basal medium	1.0 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
Basal media	216.5 mL
Noggin	1.0 mL
EGF	1.0 mL
FGF-10	1.0 mL
FGF-7	1.0 mL
A 83-01	0.1 mL
SB 202190	0.1 mL
Nicotinamide	2.5 mL
N-Acetyl-Cysteine	1.0 mL
Heregulin	1.0 mL
<b>Total volume</b>	<b>~250.0 mL</b>



6. Aseptically filter the complete growth medium through an 0.22  $\mu\text{M}$  PES bottle-top filter unit.
7. (Optional) Place the supplied 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.
- **Critical:** Supplement growth media immediately prior to use (during thaw, passage, and regular feeding) with ROCK Inhibitor (Y27632) to a final concentration of 10  $\mu\text{M}$ . We do not recommend storing complete growth media supplemented with Y27632. Only add ROCK Inhibitor to the volume being used, immediately prior to adding to cells.

#### Warranty and Disclaimer

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