Organoid media formulation #11

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

The following components are required for media preparation

Item	Vendor	Catalog #	Size	Website
Advanced DMEM:F12	Thermo Fisher	12634028	500 mL	thermofisher.com
HEPES	Thermo Fisher	15630080	100 mL	thermofisher.com
B-27 Supplement w/o VitA	Thermo Fisher	12587-010	10 mL	thermofisher.com
N2	Thermo Fisher	17502-048	5 mL	thermofisher.com
L-Glutamine	ATCC	30-2214	100 mL	atcc.org
A 83-01	Bio-techne	2939	10 mg	bio-techne.com
EGF	Bio-techne	236-EG	200 µg	bio-techne.com
HGF	Bio-techne	294-HGN	100 µg	bio-techne.com
IGF	Bio-techne	291-G1	200 µg	bio-techne.com
17-ß Estradiol	Bio-techne	2824	100 mg	bio-techne.com
Noggin	Bio-techne	6057-NG	100 µg	bio-techne.com
SB 202190	Bio-techne	1264	10 mg	bio-techne.com
Nicotinamide	LKT Labs	N3310	50 g	lktlabs.com
N-acetyl cysteine	LKT Labs	A0918	10 g	lktlabs.com
HA-R-Spondin1-Fc 293T (RSPO1) Conditioned Media	For each 500 mL of complete organoid 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://www.bio-techne.com/datasheet-pdf?src=rnd&pdf=3710-001-01.pdf			

Media preparation procedure

1. Thaw B-27 w/o VitA and L-Glutamine on ice or in a refrigerator at 2-8°C.
   Aliquot into working volumes and freeze. Do not re-freeze/thaw multiple times.

2. Briefly centrifuge the vials containing the A 83-01, EGF, HGF, IGF, Noggin, 17-ß Estradiol, and SB 202190 to ensure the material is at the bottom of the vial.

3. Aseptically reconstitute the following components according to the manufacturer's instructions in the recommended buffer: A 83-01, EGF, HGF, IGF, Noggin, 17-ß Estradiol, and SB 202190. We recommend incubating in buffer for 15 minutes at room temperature.

4. Aseptically weigh and prepare working solutions of Nicotinamide and N-Acetyl Cysteine in sterile water. If N-Acetyl Cysteine is difficult to dissolve, periodic vortexing and incubation in a 37.0°C water bath can help the material enter solution.

5. Aseptically prepare the complete growth medium formulation:

Item	Final Concentration
Advanced DMEM:F12	N/A
HEPES	10 mM
L-Glutamine	2 nM
B-27 w/o VitA	1X
N2	1X
RSPO1 CM	10%
A83-01	500 nM
17-β Estradiol	10 nM
EGF	50 ng/mL
HGF	20 ng/mL
IGF	40 ng/mL
N-Acetyl Cysteine	1.25 mM
Nicotinamide	10 mM
Noggin	100 ng/mL
SB202190	10 µM

6. Once prepared, store complete medium at 2-8°C in the dark. Do not freeze and avoid extended light exposure. Discard after 4 weeks.

7. When using the medium during culture, only warm the volume required.

8. Refer to the manufacturer's documentation for appropriate storage conditions and duration of components once in solution.

Notes

- Purity and activity levels of the various components can change from lot-lot. Refer to lot specific CoAs to ensure equivalent quality when using a new lot of material.

- We do not recommend deviating from the formulation or substituting components from different vendors.

- We recommend that solutions are prepared on the same day they are used. If the solutions must be stored, aliquot and freeze at -80°C or below and use within 30 days. Once reconstituted the components will lose activity over time and this can negatively affect performance of the medium.

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