Organoid media formulation #4

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	Thermo Fisher	17504-044	10 mL	thermofisher.com
L-Glutamine	ATCC	30-2214™	100 mL	atcc.org
Dimethyl sulfoxide (DMSO)	ATCC	4-X™	25 mL	atcc.org
Noggin	Bio-techne	6057-NG	100 µg	bio-techne.com
EGF	Bio-techne	236-EG	200 µg	bio-techne.com
Gastrin	Bio-techne	3006	1 mg	bio-techne.com
SB202190	Bio-techne	1264	10 mg	bio-techne.com
A83-01	Bio-techne	2939	10 mg	bio-techne.com
N2-MAX	Bio-techne	AR009	5 mL	bio-techne.com
Prostaglandin E_2 (PGE_2)	Bio-techne	2296	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, 100 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			

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

Media preparation procedure

1. Thaw B-27, N2-MAX, 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 Noggin, EGF, Gastrin, PGE2, SB202190, and A83-01 to ensure the material is at the bottom of the vial.

3. Aseptically reconstitute the following components: Noggin, EGF, PGE2, Gastrin, SB202190 and A83-01 according to the manufacturer's instructions in the recommended buffer listed in the table below. We recommend incubating in buffer for 15 minutes at room temperature.

Item	Size	Buffer	Volume of Buffer	Final Concentration
Noggin	100 µg	Advanced DMEM:F12	1.0 mL	100 µg/mL
EGF	200 µg	Advanced DMEM:F12	2.0 mL	100 µg/mL
Gastrin	1 mg	Advanced DMEM:F12	4.7 mL	100 µM
A83-01	10 mg	DMSO	0.95 mL	25 mM
SB202190	10 mg	DMSO	1.2 mL	25 mM
PGE_2	10 mg	DMSO	28.3 mL	100 µM

4. Aseptically weigh and prepare working solutions of Nicotinamide and N-Acetyl Cysteine in the recommended buffer. 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.

Item	Weight	Buffer	Volume of Buffer	Final Concentration
Nicotinamide	5 g	Advanced DMEM:F12	41.0 mL	1 M
N-Acetyl Cysteine	2.5 g	Advanced DMEM:F12	61.0 mL	250 mM

5. Prepare the complete growth medium formulation (makes 500 mL)

Item	Volume	Final Concentration
Advanced DMEM:F12	366.0 mL	N/A
HEPES	5.0 mL	10 mM
L-Glutamine	5.0 mL	2 nM
B-27	10.0 mL	1X
N2-MAX	5.0 mL	1X
Noggin	0.5 mL	100 ng/mL
EGF	0.25 mL	50 ng/mL
Gastrin	50.0 µL	10 nM
A83-01	10.0 µL	500 nM
SB202190	100.0 uL	3 uM
PGE_2	50.0 uL	10 nM
Nicotinamide	5.0 mL	10 mM
N-Acetyl Cysteine	2.5 mL	1.25 mM
RSPO1 conditioned media	100 mL	20%

6. Once prepared, store complete medium at 2-8°C in the dark. Do not freeze and avoid extended exposure to light. Label with date of preparation and discard after 30 days.

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

8. Refer to the manufacturer's documentation for appropriate storage conditions and stability of individual components once reconstituted.

Notes

- Purity and activity levels of the various components can change from lot-lot. Refer to the manufacturer's Certificates of Analysis 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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