Organoid media formulation #12

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	Thermo Fisher	17504-044	10 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
CHIR 99021	Bio-techne	4423	10 mg	bio-techne.com
FGF-2	Bio-techne	233-FB	10 µg	bio-techne.com
FGF-10	Bio-techne	345-FG	10 µg	bio-techne.com
Forskolin	Bio-techne	1099	10 mg	bio-techne.com
Prostaglandin E_2 (PGE2)	Tocris	2296	25 µg	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 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, CHIR 99021, FGF-2, FGF-10, Forskolin, PGE2, and Noggin 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, CHIR 99021, FGF-2, FGF-10, Forskolin, PGE2, and Noggin. 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 Supplement	1X
RSPO1 CM	10%
CHIR 99021	3 µM
EGF	50 ng/mL
FGF-2	2.5 ng/mL
FGF-10	10 ng/mL
Forskolin	1 µM
N-Acetyl Cysteine	1.25 mM
Nicotinamide	10 mM
Noggin	100 ng/mL
Prostaglandin E_2	1 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.

Warranty and Disclaimer

ATCC® products are warranted for 30 days from the date of shipment, and is valid only if the product is stored and used according to the information included on this product information sheet. This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. ATCC does not warrant that such information has been confirmed to be accurate. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product.