



Organoid media formulation #1

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
Dimethyl sulfoxide (DMSO)	ATCC	4-X™	25 mL	atcc.org
Noggin	Bio-technie	6057-NG	100 µg	bio-technie.com
EGF	Bio-technie	236-EG	200 µg	bio-technie.com
Gastrin	Bio-technie	3006	1 mg	bio-technie.com
SB202190	Bio-technie	1264	10 mg	bio-technie.com
A83-01	Bio-technie	2939	10 mg	bio-technie.com
Nicotinamide	LKT Labs	N3310	50 g	lktlabs.com
N-acetyl cysteine	LKT Labs	A0918	10 g	lktlabs.com

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 Noggin, EGF and Gastrin, SB202190 and A83-01 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: Noggin, EGF and Gastrin, SB202190 and A83-01. We recommend incubating in buffer for 15 minutes at room temperature.

Item	Size	Buffer	Volume of buffer	Final Concentration
Noggin	100 µg	Basal medium	1.0 mL	100 µg/mL
EGF	200 µg	Basal medium	2.0 mL	100 µg/mL
Gastrin	1 mg	Basal medium	4.7 mL	100 µM
SB202190	10 mg	DMSO	0.6 mL	50 mM
A83-01	10 mg	DMSO	0.95 mL	25 mM

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.

Item	Weight	Buffer	Volume of buffer	Final Concentration
Nicotinamide	5 g	Basal medium	41.0 mL	1 M
N-Acetyl Cysteine	2.5 g	Basal medium	61.0 mL	250 mM



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

Item	Volume	Final Concentration
Advanced DMEM:F12	471.0 mL	N/A
HEPES	5.0 mL	10 mM
L-Glutamine	5.0 mL	2 nM
B-27	10.0 mL	1X
Noggin	0.5 mL	100 ng/mL
EGF	0.25 mL	50 ng/mL
Gastrin	50.0 µL	10 nM
SB202190	100.0 µL	10 µM
A83-01	10.0 µL	500 nM
Nicotinamide	5.0 mL	10 mM
N-Acetyl Cysteine	2.5 mL	1.25 mM

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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