



## Organoid media formulation #1

### Components required

| Item                   | Manufacturer       | Catalog # | Storage        |
|------------------------|--------------------|-----------|----------------|
| Organoid Growth Kit 1A | ATCC               | ACS-7100  | -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 Sci. | 12634028  | 2-8°C          |
| HEPES                  | Thermo Fisher Sci. | 15630080  | 2-8°C          |
| B-27 Supplement        | Thermo Fisher Sci. | 17504-044 | -20°C or below |

Refer to manufacturer documentation for expiration dates and safe handling information.

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

| Item                | Volume   |
|---------------------|----------|
| Advanced DMEM:F12   | 240 mL   |
| HEPES               | 2.5 mL   |
| L-Glutamine         | 2.5 mL   |
| B-27                | 5.0 mL   |
| <b>Total volume</b> | 250.0 mL |

3. Briefly centrifuge the vials in the Organoid Growth Kit to ensure the contents are at the bottom of the vials.
4. Aseptically reconstitute the components in the indicated buffer. After adding buffer, 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-7202  | Supplemented basal medium | 1.0 mL           |
| Gastrin           | ACS-7208  | Supplemented basal medium | 0.5 mL           |
| Nicotinamide      | ACS-7214  | Supplemented basal medium | 2.5 mL           |
| N-Acetyl-Cysteine | ACS-7215  | Supplemented basal medium | 1.0 mL           |
| SB 202190         | ACS-7211  | DMSO                      | 0.1 mL           |
| A 83-01           | ACS-7209  | DMSO                      | 0.1 mL           |



**Note: Once reconstituted components should be used immediately. Do not store reconstituted components.**

5. Aseptically prepare the complete growth medium formulation by combining the reconstituted prepared kit components with the supplemented basal medium.

| Item                      | Volume           |
|---------------------------|------------------|
| Supplemented basal medium | 244 mL           |
| Noggin                    | 1.0 mL           |
| EGF                       | 1.0 mL           |
| Gastrin                   | 0.5 mL           |
| SB 202190                 | 0.1 mL           |
| A 83-01                   | 0.1 mL           |
| Nicotinamide              | 2.5 mL           |
| N-Acetyl-Cysteine         | 1.0 mL           |
| <b>Total volume</b>       | <b>~250.0 mL</b> |

6. Aseptically filter the complete growth medium through an 0.22  $\mu$ M PES bottle-top filter unit.
7. (Optional) Place the supplied sticker 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.

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