

CELLiST™ FEED2 Medium

| Name | Model number | Packaging | Contents |
|---------------------|--------------|----------------------|----------|
| CELLiST™ Feed media | FEED2 | 0.2 L Aluminum Pouch | 22.0 g |

Properties:

- Chemically-defined, animal origin-free, with no proteins or growth factors added.
- Does NOT contain hydrolysates, extracts or other undefined components.
- Does NOT contain thymidine or hypoxanthine.
- Does NOT contain L-glutamine sources.
- Does NOT contain sodium bicarbonate.
- Does NOT contain glucose. When adding glucose, it is recommended to add 70–100 g/L, depending on feeding conditions.

Storage conditions:

Before liquid preparation, store powder media in a dark and refrigerated place (2–8°C), away from high humidity. After liquid preparation, store in a dark and refrigerated place (2–8°C) and use within 1 month.

Instructions for preparation of liquid medium:

1. Prepare a suitable container and stir bar (magnetic bar). To ensure sufficient stirring, we recommend a container with a capacity of about 2–3 times the total prepared volume. When preparing on a weight basis, measure the weight of the container and the stir bar.
2. Fill the container with about 70% volume (140 mL) of cell culture-grade water (room temperature).
3. The total amount of this pouch (22.0 g) should be added to the container. Place a small amount of cell culture-grade water in the pouch to wash the remaining product into the container.
4. Add glucose as needed.
5. Stir for about 30 minutes.
6. Add 1.04 mL of 8N NaOH solution.
7. Stir for at least 30 minutes or until the powder is completely dissolved.
8. Make sure the pH is between 6.6-7.0. If the pH is lower than 6.6, add 8N NaOH solution to adjust to 6.6-7.0. If the pH is higher than 7.0, the stirring time should be extended to allow complete dissolution of the medium components.
9. Adjust to the final volume (200 mL) with cell culture-grade water and stir until the solution is clear for approximately 15 minutes.
10. Under aseptic conditions (e.g., in biosafety cabinet), filter sterilization using a filter with a pore size of 0.20–0.22 µm.
11. Store in a refrigerated (2–8°C), dark environment until use.

Use:

- This product is a cell culture medium used for research applications. Do not use it for any other purpose.
- For use in manufacturing, and for any other inquiries, please contact the following:

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Recommended fed-batch culture conditions in shake flasks:

- (1) Prepare liquid media following CELLiST™ media preparation instructions.
- (2) Inoculate cells to 30 mL of basal media at 0.5×10^6 cells/mL in 125 mL vented cap shake flask.
- (3) After inoculation, perform measurements to confirm appropriate cell density and viability.

Recommended feeding strategy:

- Feeding volumes in the table below are stated as % (v/v) from the total initial volume.
- It is recommended to start feeding from Day 3, with daily additions of feed. If it is difficult to perform daily feed additions, then every other day is possible (see table below).
- Feeding volume depends on cell line characteristics. For high-productivity cell lines (titer >5 g/L), larger feeding volume may be required. See table below for information.
- Make sure to measure glucose concentration daily and top up glucose separately to maintain a concentration of 2-6 g/L (glucose consumption rate varies depending on cell line).
- For getting the most out of your cell line, it is recommended to optimize feeding volume by testing multiple feeding volumes (e.g. 2%, 4%, 6% every other day).

Example for recommended feed addition:

| Additives | Cell type | Cultivation day | | | | | | | | | | | | | |
|--------------|----------------------------|---------------------------------------|---|----|----|----|----|----|----|----|----|----|----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| FEED2 (%v/v) | High titer (≥ 5 g/L) | | | 3% | 3% | 3% | 3% | 3% | 3% | 3% | 3% | 3% | 3% | 3% | |
| FEED2 (%v/v) | Low titer (<5 g/L) | | | 2% | 2% | 2% | 2% | 2% | 2% | 2% | 2% | 2% | 2% | 2% | |
| Glucose | | Measure daily and maintain at 2-6 g/L | | | | | | | | | | | | | |

| Additives | Cell type | Cultivation day | | | | | | | | | | | | | |
|--------------|----------------------------|---------------------------------------|---|----|---|----|---|----|---|----|----|----|----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| FEED2 (%v/v) | High titer (≥ 5 g/L) | | | 6% | | 6% | | 6% | | 6% | | 6% | | | |
| FEED2 (%v/v) | Low titer (<5 g/L) | | | 4% | | 4% | | 4% | | 4% | | 4% | | | |
| Glucose | | Measure daily and maintain at 2-6 g/L | | | | | | | | | | | | | |