



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 $(2-8^{\circ}C)$, away from high humidity. After liquid preparation, store in a dark and refrigerated place $(2-8^{\circ}C)$ 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.
- 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:

AJINOMOTO CELLIST KOREA CO., INC.

CELLiST Solution Center (CSC) #1403~1405, Songdo AT Center, 70 Songdogwahak-ro, Yeonsu-gu, Incheon, Republic of Korea Tel : +82-32-210-2603 Fax : +82-32-210-2604

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 x 10⁶ 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).

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													

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)			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													

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