

CELLiST™ F7 Feed Medium

Product Type	Product Name	Packaging	Contents
Feed Medium	CELLiST™ F7	0.2 L Aluminum Pouch	24.0 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:

Please store the powdered medium before liquid preparation in a refrigerator (2–8°C) in a dark place, avoiding high humidity.

After preparing the liquid, store it in a refrigerator (2–8°C) in a dark place and use it within one month.

Instruction for preparation of liquid medium:

Table 1: Various parameters for preparation of 0.2 L of feed medium (with and without glucose addition):

Glucose concentration	Powder weight	Glucose weight	8N NaOH solution to be added	Total added water	pH*	Osmotic pressure* (5-fold dilution)	Total solution weight	Specific gravity (Room temperature)
0 g/L	24.0 g	0 g	2.0 mL (2.66 g)	181 mL (181 g)	6.6–7.0	205–225 mOsm/kg	208 g	1.04
70 g/L	24.0 g	14 g	2.2 mL (2.93 g)	173 mL (173 g)	6.6–7.0	280–300 mOsm/kg	214 g	1.07

*Reference value

1. Prepare a suitable container and stir bar, ideally with a capacity 2–3 times the final volume. If preparing by weight, measure the weights of both the container and stir bar.
2. Fill the container with about 70% volume (140 mL) of cell culture-grade water (room temperature).
3. Add the entire contents of the pouch (24.0 g) to the container, rinsing any residue with a small amount of cell culture grade water.
Dissolve the product promptly after opening to prevent moisture absorption.
4. Add glucose as needed.
5. Add cell culture grade water (room temperature) until the volume reaches about 90–95% of the final volume.
6. Stir well for about 30 minutes, adjusting the stirring speed so that the bottom of the whirlpool reaches the stir bar. Avoid excessive stirring to prevent splashing.
7. Refer to Table 1 and add 8N NaOH solution. If you add a different amount of glucose than indicated in Table 1, add 8N NaOH solution while checking that the pH is between 6.6 and 7.0.
8. Stir well for at least 30 minutes until completely dissolved, adjusting the stirring speed so that the bottom of the whirlpool reaches the stir bar. Avoid excessive stirring to prevent splashing.
9. Confirm that the pH is between 6.6 and 7.0.
If the pH is < 6.6, adjust it to between 6.6 and 7.0 using 8N NaOH solution.
If the pH is > 7.0, dissolution may be insufficient, so extend the stirring time.
10. Adjust the final volume to 200 mL with cell culture grade water and stir for about 15 minutes until uniform. Alternatively, adjust by weight according to Table 1.
11. Check the pH and osmolality. Measure osmolality after 5-fold dilution.
12. Filter the media under aseptic conditions using a filter with a pore size of 0.20–0.22 µm.
13. Store in a refrigerator (2–8°C) in a dark place 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

- (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).
- To maximize productivity, it is recommended to evaluate multiple addition amounts (e.g., 2%, 4%, 6% every other day) and optimize the amount to be added.

Example for recommended feed addition:

Feed	Cell type	Cultivation day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
F7 (%v/v)	High titer (≥ 5 g/L)			3%	3%	3%	3%	3%	3%	3%	3%	3%	3%	3%	
F7 (%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													

Feed	Cell type	Cultivation day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
F7 (%v/v)	High titer (≥ 5 g/L)			6%		6%		6%		6%		6%			
F7 (%v/v)	Low titer (<5 g/L)			4%		4%		4%		4%		4%			
Glucose		Measure daily and maintain at 2-6 g/L													