

Eden F100bS Feed Medium

Product Name: F100bS

User Manual

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Description

Eden F100bS feed medium is a chemical-defined, protein-free, and animal-free, highly concentrated feed medium specifically designed for fed-batch process, and perfusion process of Chinese Hamster Ovary (CHO) cells. The medium enables the excellent growth performance of many CHO cells and the high-level expression of recombinant proteins and therapeutic antibodies, in conjunction with the Eden series basal medium (Refer to the "Related Product" section).

Application

Eden F100bS feed medium is suitable for general culture with CHO cell lines such as CHO-K1, CHOS, CHOZN, CHO DG44 and CHO DUX11.

This product is intended for research or further manufacturing in the bio-manufacturing industry, but not for human or therapeutic use.

Composition

The IP rights of Eden F100bS feed medium formulation are owned by Shanghai BioEngine Sci-Tech Co., Ltd.

This medium contains:

Amino acids.

Not contain:

- D-glucose, P188, Hydrolysates, cytokines,
 L-glutamine, antibiotics, HEPES, phenol red and nucleosides.
- \boxtimes Raw materials from animal sources.

Storage

- Store medium at 2-8°C, away from light.
- Once opened, the powder medium should be stored protected from moisture in a tightly sealed container.
- Do not use it after the expiration date or being damped.

Reconstitution of Powder Medium

Reconstitution by constant volume

Table 1 shows the preparation of Eden F100bS feed medium ^[1].

Ingredients	Concentration
Eden F100bS feed medium	88.00 g/L ^[2]

Table 1. Preparation of Eden F100bS feed medium

- Weigh 80% water of the final volume into the preparation container using pure water, ultrapure water, or water for injection at 20-30°C. Mix thoroughly (Power per Volume (P/V) >10 W/m³) without creating air bubbles.
- Accurately weigh the corresponding mass of Eden F100bS feed medium at a concentration of 88.00 g/L, and add it into the preparation container of (1), and stir well for 20 minutes.
- Slowly adjust to pH 11.0-12.0 with 10 mol/L sodium hydroxide solution or sodium hydroxide powder. Stir for 25 minutes.
- Quantify with preparation water to reach 100% of the volume. Stir for 10 minutes.

- Pass the Eden medium solution through a pore size of 0.22 or 0.2 µm sterile filter membrane, such as PES, using a pulse pump or compressed air (3-15 psi).
- 6) Use the prepared medium liquid immediately or store it in glass bottles, PET storage bottles, or disposable storage bags with an oxygen barrier membrane in a dark environment of 2~8°C. The reconstituted medium is stable for 2 months.

Note:

^[1] The above parameters (such as stirring time and P/V) are set for small-scale liquid preparation. Adjust these parameters for large-scale preparation based on container capacity to ensure full dissolution of dry powder.

^[2] The "g/L" unit denotes volumetric concentration (solute mass/solution volume).

Reconstitution by constant weight

Table 2 shows the preparation of Eden F100bS feed medium ^[3].

Eden F100bS feed medium 83	8.72 g/kg ^[4]

Table 2. Preparation of Eden F100bS basal medium

- Weigh 80% water of the final weight into the preparation container using pure water, ultrapure water, or water for injection at 20-30°C. Mix thoroughly (Power per Volume (P/V) >10 W/m³) without creating air bubbles.
- Accurately weigh the corresponding mass of Eden F100bS feed medium at a concentration of 83.72 g/kg, and add it into the preparation container of (1), and stir well for 20 minutes.
- Slowly adjust to pH 11.0-12.0 with 10 mol/L sodium hydroxide solution or sodium hydroxide powder. Stir for 25 minutes.
- Quantify with preparation water to reach 100% of the weight. Stir for 10 minutes.

- Pass the Eden medium solution through a pore size of 0.22 or 0.2µm sterile filter membrane, such as PES, using a pulse pump or compressed air (3-15 psi).
- 6) Use the prepared medium liquid immediately or store it in glass bottles, PET storage bottles, or disposable storage bags with an oxygen barrier membrane in a dark environment of 2~8°C. The reconstituted medium is stable for 2 months.

Note:

^[3] The above parameters (such as stirring time, and P/V) are set for small-scale liquid preparation. Adjust these parameters for large-scale preparation based on container capacity to ensure full dissolution of dry powder.

^[4] The "g/kg" unit denotes mass concentration (solute mass/solution mass).

Specifications of final liquid medium

Test	Unit	Specification		
рН		11.0 – 12.0		
Osmolality	mOsm/kg	1100 – 1600		
Turbidity	NTU	< 4.00		

Table 3. Specifications of final liquid medium

Fed-batch Culture

<u>Culture system</u>

Shake flask or spin tube.

Culture conditions

Incubate at 37° C in a humidified atmosphere of 5-8% CO₂ in air on an orbital shaker platform (amplitude: 50 mm) rotating at 115-135 rpm (shake flask) or 215-225 rpm (spin tube).

Feed strategy

Condition Max VCD in process	Feed Medium ^[6]	D3	D5	D7	D9	D10	D11	D12	D13
<2×10 ⁷ cells/mL	Feed Medium a (%)	4	4	4-5	4-5	/	4-5	1	3-4
<2×10 ⁷ cens/mL	Feed Medium b (%) Feed Medium a: Feed Medium b= 10:1 (v/v)								
0.0	Feed Medium a (%)	4	4	5-6	5-6	1	4-5	1	4
2~3×10 ⁷ cells/mL	Feed Medium b (%) Feed Medium a: Feed Medium b= 10:1 (v/v)								
	Feed Medium a (%)	4	4-5	6	3-4	3-5	3-5	3-5	3-4
>3×10 ⁷ cells/mL	Feed Medium b (%)Feed Medium a: Feed Medium b= 10:1 (v/v)								

Table 4. Recommended feed strategy

- Incubate CHO cells, in the mid-log phase of growth with >90% viability, into a shake flask/spin tube at a seeding density of 0.5-0.7×10⁶ viable cells/mL.
- 2) Follow the suggested feed strategy ^[5] in Table 4.
- Ensure the residual glucose concentration is maintained above 2 g/L during the fed-batch process.
- Harvest the cells on day 14 or when viability falls below 50%.

Note:

^[5] (a) Select the feeding strategy based on the maximum viable cell density (VCD) of the original process and previous cell growth performance. (b) Reduce the feed volume appropriately in the temperature-shift fed-batch process. (c) Advance the feed time when the seeding density is increased. (d) Follow the optimal feed strategy when using the Eden series medium.

^[6] The feed medium a and feed medium b volumes can be calculated by initial culture volume. Check the "Related Product" section or contact BioEngine technical support department for optimal combinations of Eden serial Media.

Perfusion Culture

Culture system

Spin tube.

Culture conditions

Incubate at 37°C in a humidified atmosphere of 5-8% CO₂ in air on an orbital shaker platform (amplitude: 50 mm) rotating at 250-300 rpm.

Perfusion medium preparation

Prepare the required volume of perfusion medium consisting of 95% Eden series basal medium and feed medium a (Refer to the "Related Product" section).

Perfusion strategy

- Inoculate CHO cells, in mid-log phase of growth with >90% viability, into a spin tube with a seeding density of 0.4-0.6×10⁶ viable cells/mL.
- Start the perfusion culture when the VCD reaches 3-5×10⁶ cells/mL.
- Harvest CHO cells through centrifugation at 100×g for 5-10 minutes and resuspend in the perfusion medium, maintaining a constant working volume every day. Additionally, add 0.5% (v/v) Eden F100bS feed medium.
- When the VCD reaches 10-20×10⁶ cells/mL or 50-60×10⁶ cells/mL, increase the proportion of feed medium a in perfusion medium and adjust the volume of Eden F100bS feed medium accordingly.
- Ensure the residual glucose concentration is maintained above 2 g/L during the perfusion process.

Related Product							
Product Name	Туре	Cat. No.	Form	Size	Packaging	Note	
Eden B100S	Basal medium	EXP0116401	Powder	200 L	Bag	Suitable for general culture with CHO cell lines such as CHO-K1,	
		EXP0116402	Powder	10 L	Bag	CHOS, CHO DG44 and CHO DUX11.	
Eden F100aS	Feed medium a	EXP0116501	Powder	20 L	Bag	Add 4-8 mM L-glutamine in basal medium for non-GS CHO cell	
		EXP0116502	Powder	1 L	Bag	applications.	
Eden F100bS	Feed medium b	EXP0116601	Powder	20 L	Bag	Add cytokines in Basal medium or Feed medium a for	
		EXP0116602	Powder	1 L	Bag	cytokines-depended CHO cell applications.	



Scan the QR code for more details about Eden CHO CD Media.

Stay tuned for more updates. Tel: 86-21-68582660 Web: <u>www.bio-engine.com.cn/EN</u>

