

# **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.
- 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 <sup>[1]</sup>.

Ingredients	Concentration
Eden F100bS feed medium	88.00 g/L <sup>[2]</sup>

Table 1. Preparation of Eden F100bS feed medium

- 1) 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/m<sup>3</sup>) without creating air bubbles.
- 2) 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.
- 3) Slowly adjust to pH 11.0-12.0 with 10 mol/L sodium hydroxide solution or sodium hydroxide powder. Stir for 25 minutes.
- 4) Quantify with preparation water to reach 100% of the volume. Stir for 10 minutes.

- 5) Pass the Eden medium solution through a pore size of 0.22 or 0.2  $\mu\text{m}$  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:

<sup>[1]</sup> 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.

<sup>[2]</sup> 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 <sup>[3]</sup>.

Ingredients	Concentration
Eden F100bS feed medium	83.72 g/kg <sup>[4]</sup>

Table 2. Preparation of Eden F100bS basal medium

- 1) 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/m<sup>3</sup>) without creating air bubbles.
- 2) 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.
- 3) Slowly adjust to pH 11.0-12.0 with 10 mol/L sodium hydroxide solution or sodium hydroxide powder. Stir for 25 minutes.
- 4) Quantify with preparation water to reach 100% of the weight. Stir for 10 minutes.

- 5) Pass the Eden medium solution through a pore size of 0.22 or 0.2  $\mu\text{m}$  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:

<sup>[3]</sup> 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.

<sup>[4]</sup> The “g/kg” unit denotes mass concentration (solute mass/solution mass).

### Specifications of final liquid medium

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

Table 3. Specifications of final liquid medium

## **Fed-batch Culture**

### **Culture system**

Shake flask or spin tube.

### **Culture conditions**

Incubate at 37°C in a humidified atmosphere of 5-8% CO<sub>2</sub> 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	Feed Medium <sup>[6]</sup>	D3	D5	D7	D9	D10	D11	D12	D13
Max VCD in process	Feed Medium a (%)	4	4	4-5	4-5	/	4-5	/	3-4
	Feed Medium b (%)	Feed Medium a: Feed Medium b= 10:1 (v/v)							
2~3×10 <sup>7</sup> cells/mL	Feed Medium a (%)	4	4	5-6	5-6	/	4-5	/	4
	Feed Medium b (%)	Feed Medium a: Feed Medium b= 10:1 (v/v)							
>3×10 <sup>7</sup> cells/mL	Feed Medium a (%)	4	4-5	6	3-4	3-5	3-5	3-5	3-4
	Feed Medium b (%)	Feed Medium a: Feed Medium b= 10:1 (v/v)							

Table 4. Recommended feed strategy

- 1) Incubate CHO cells, in the mid-log phase of growth with >90% viability, into a shake flask/spin tube at a seeding density of 0.5-0.7×10<sup>6</sup> viable cells/mL.
- 2) Follow the suggested feed strategy <sup>[5]</sup> in Table 4.
- 3) Ensure the residual glucose concentration is maintained above 2 g/L during the fed-batch process.
- 4) Harvest the cells on day 14 or when viability falls below 50%.

Note:

<sup>[5]</sup> (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.

<sup>[6]</sup> 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% CO<sub>2</sub> 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

- 1) Inoculate CHO cells, in mid-log phase of growth with >90% viability, into a spin tube with a seeding density of 0.4-0.6×10<sup>6</sup> viable cells/mL.
- 2) Start the perfusion culture when the VCD reaches 3-5×10<sup>6</sup> cells/mL.
- 3) 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.
- 4) When the VCD reaches 10-20×10<sup>6</sup> cells/mL or 50-60×10<sup>6</sup> cells/mL, increase the proportion of feed medium a in perfusion medium and adjust the volume of Eden F100bS feed medium accordingly.
- 5) Ensure the residual glucose concentration is maintained above 2 g/L during the perfusion process.

## Related Product

Product Name	Type	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, CHOS, CHO DG44 and CHO DUX11.
		EXP0116402	Powder	10 L	Bag	
Eden F100aS	Feed medium a	EXP0116501	Powder	20 L	Bag	Add 4-8 mM L-glutamine in basal medium for non-GS CHO cell applications.
		EXP0116502	Powder	1 L	Bag	
Eden F100bS	Feed medium b	EXP0116601	Powder	20 L	Bag	Add cytokines in Basal medium or Feed medium a for cytokines-dependent CHO cell applications.
		EXP0116602	Powder	1 L	Bag	



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

Stay tuned for more updates.

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