Eden B601S Basal Medium

Product Name: B601S

User Manual

Contents

Description

Eden B601S basal medium is a chemical-defined, protein-free, and animal-free basal medium specifically designed for cryopreservation, recovery, subculture, 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 feed medium (Refer to the “Related Product” section).

Application

Eden B601S basal medium is suitable for general culture with CHO cell lines such as CHO-K1, CHOS, 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 B601S basal medium formulation are owned by Shanghai BioEngine Sci-Tech Co., Ltd.

This medium contains:

☑ Carbohydrates, amino acids, vitamins, bulk salts, trace elements, nucleosides.

☑ 6.30 g/L D-glucose，1.00 g/L P188.

Not contain:

☒ Hydrolysates, cytokines, L-glutamine, antibiotics, HEPES and phenol red.

☒ 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 B601S basal medium [1].

Ingredients	Concentration
Eden B601S basal medium	22.80 g/L [2]
Sodium bicarbonate	2.20 g/L

Table 1. Preparation of Eden B601S basal medium

1) Weigh 90 % 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^3) without creating air bubbles.

2) Accurately weigh the corresponding mass of Eden B601S basal medium at a concentration of 22.80 g/L, and add it into the preparation container of (1), and stir well for 15 minutes.

3) Slowly adjust to pH 6.0-6.5 with 5-10 mol/L sodium hydroxide solution. Stir for 15 minutes.

4) Weigh 2.20 g/L of sodium bicarbonate powder, add it slowly near the liquid level in the container, and stir for 10 minutes. Quantify with preparation water to reach 100% of the volume. Stir for 10 minutes.

5) Adjust to pH 7.0-7.4 with sodium hydroxide or hydrochloric acid solution.

6) 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).

7) 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℃. The reconstituted medium is stable for 6 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

Ingredients	Concentration
Eden B601S basal medium	21.99 g/kg [4]
Sodium bicarbonate	2.12 g/kg

Table 2. Preparation of Eden B601S basal medium

Table 2 shows the preparation of Eden B601S basal medium [3].

1) Weigh 90% water of the final weight into the preparation container using pure water, ultrapure water, or water for injection at 20-30℃. Mix thoroughly (Power per Volume (P/V) >10 W/m^3) without creating air bubbles.

2) Accurately weigh the corresponding mass of Eden B601S basal medium at a concentration of 21.99 g/kg, and add it into the preparation container of (1), and stir well for 15 minutes.

3) Slowly adjust to pH 6.0-6.5 with 5-10 mol/L sodium hydroxide solution. Stir for 15 minutes.

4) Weigh 2.12 g/kg of sodium bicarbonate powder, add it slowly near the liquid level in the container, and stir for 10 minutes. Quantify with preparation water to reach 100% of the weight. Stir for 10 minutes.

5) Adjust to pH 7.0-7.4 with sodium hydroxide or hydrochloric acid solution.

6) 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).

7) 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℃. The reconstituted medium is stable for 6 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
pH		7.0 – 7.4 [5]
Osmolality	mOsm/kg	280 – 320
Turbidity	NTU	< 4.00

Table 3. Specifications of final liquid medium

Note:

[5] The pH buffer system of the product is carbon dioxide-sodium bicarbonate. The final pH value should be strictly controlled within the specific range outlined in Table 3. The following operations, such as prolonged reconstitution time or aeration in the bioreactor without pH control, can result in a gradual pH increase. There is a risk of metal ion precipitation when the pH value exceeds the upper limit.

Cryopreservation

1) Harvest CHO cells, in the mid-log phase of growth with >90% viability by centrifugation at 100×g for 5-10 minutes. Reserve supernatant (the conditioned medium) for cryopreservation medium preparation.

2) Prepare cryopreservation medium with 41% conditioned medium, 41% Eden B601S basal medium, and 8% DMSO on the day of use.

3) Resuspend cells in cryopreservation medium to a final viable cell density of >1.0×10^7 cells/mL or as required.

4) Dispense aliquots of the cell suspension into cryovials.

5) Achieve cryopreservation in an automated or manual controlled rate freezing apparatus (0.5-1°C decrease per minute is suggested).

6) Transfer frozen cells to liquid nitrogen storage.

Cell Recovery

1) Rapidly thaw frozen cells (<1 minute) in an automated apparatus or 37°C water bath.

2) Harvest the cells by centrifugation at 100×g for 5-10 minutes and discard the supernatant.

3) Resuspend cells by fresh Eden B601S basal medium to a viable cell density of 0.5-1.0×10^6 cells/mL in a 125mL shake flask or a 50mL spin tube.

4) Incubate at 37°C in a humidified atmosphere of 5-8% CO_2 in air on an orbital shaker platform (amplitude: 50 mm) rotating at 115-135 rpm (shake flask) or 215-225 rpm (spin tube).

5) Maintain a viable cell density of 0.5-1×10^6 cells/mL for the first two passages following recovery. Then, resume the subculture schedule.

Subculture Cells

1) Determine viable cell density using a Cell Counter.

2) Ensure that the viable cell density is $\geqslant1\times10^6$ cells/mL, viability is ⩾90%, and the growth rate is in mid-logarithmic phase prior to subculturing.

3) Calculate the volume of cell culture and medium necessary to seed at 0.4-0.6×10^6 viable cells/mL in a shake flask or a spin tube.

4) Incubate at 37°C in a humidified atmosphere of 5-8% CO_2 in air on an orbital shaker platform (amplitude: 50 mm) rotating at 115-135 rpm (shake flask) or 215-225 rpm (spin tube).

5) For optimal performance and cell growth, dilute the cells at a seeding density of 0.4-0.6×10^6 viable cells/mL every 2-4 days with fresh Eden B601S basal medium or lab subculture schedule.

Adaptation of CHO Cells to Eden Medium

Adapting CHO cells to Eden Medium before fed-batch or perfusion culture is highly recommended. It is crucial

that cell viability should be ⩾90% and the growth rate should be in the mid-logarithmic phase before starting the adaptation process. Maintaining backup cultures is also advised until full adaptation is achieved.

Direct adaptation

1) The method is recommended for adapting CHO cells from a serum-free medium.

2) Dilute cells with 100% fresh Eden B601S basal medium to a seeding density of 0.4-0.6×10^6 viable cells/mL during subculturing (Refer to the "Subculture Cells" section).

3) Continue regular subculturing every 2-4 days or according to your lab subculture schedule until consistent growth is achieved.

Sequential adaptation

1) The method is recommended for adapting CHO cells from serum medium or partial serum-free medium.

2) Dilute cells using a 25:75 ratio of Eden B601S basal medium to the original medium to a seeding density of 0.4-0.6×10^6 viable cells/mL [6]. Subculture the CHO cells under this condition until consistent growth is achieved.

3) In each subsequent passage, gradually increase the ratio of Eden Medium to the original medium (25:75, 50:50, 75:25, 90:10, and finally 100% Eden Medium) until consistent growth is achieved.

4) Adaptation is considered complete when the culture performance (specific growth rate, double timing, viability *et al.*) in 100% Eden B601S basal medium matches that of the original culture system.

Note:

[6] If the cell growth performance does not recover after multiple subcultures, it is recommended to increase the seeding density to 1.0-1.5×10^6 viable cells/mL via sedimentation or centrifugation.

Fed-batch Culture

Culture system

Shake flask or spin tube.

Culture conditions

Incubate at 37°C in a humidified atmosphere of 5-8% CO_2 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

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 0.5-0.7×10^6 viable cells/mL.

2) Follow the suggested feed strategy [7] as outlined 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:

[7] (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.

[8] 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.

Condition Max VCD in process	Feed Medium [8]	D3	D5	D7	D9	D10	D11	D12	D13
<2×10^7 cells/mL	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*)							
2~3×10^7 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*)							
>3×10^7 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

Perfusion Culture

Culture system

Spin tube.

Culture conditions

Incubate at 37°C in a humidified atmosphere of 5-8% CO_2 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 B601S basal medium and 5% Eden series 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 0.4-0.6×10^6 viable cells/mL.

2) Start the perfusion culture when the VCD reaches 3-5×10^6 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*) feed medium b (Refer to the “Related Product” section).

4) When the VCD reaches 10-20×10^6 cells/mL or 50-60×10^6 cells/mL, increase the proportion of feed medium a in perfusion medium and adjust the volume of feed medium b 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 B601S	Basal medium	EXP0115601	Powder	200 L	Bag	Suitable for general culture with CHO cell lines such as CHO-K1, CHOS, CHO DG44 and CHO DUX11.
		EXP0115603	Powder	10 L	Bag	
Eden F602aS	Feed medium a	EXP0115701	Powder	20 L	Bag	Add 4-8 mM L-glutamine in basal medium for non-GS CHO cell applications.
		EXP0115702	Powder	1 L	Bag	
Eden F600bS	Feed medium b	EXP0108801	Powder	10 L	Bag	Add cytokines in Basal medium or Feed medium a for cytokines-depended CHO cell applications.
		EXP0108802	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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