CelliMax® Zeta02 plus Medium

Instructions for Use

Cat. No.: CPDP089 (Powder)

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Product Description

CelliMax® Zeta02 plus Medium is a chemically defined (CD) basal medium. It is free of animal-derived components and hydrolysates, and does not contain L-glutamine or phenol red. When used in combination with the CelliMax® feed medium portfolio, it supports high-density growth of various CHO cell lines and enhances protein expression.

Intended Use

This product is intended for research use and for the manufacture of biological products derived from cell culture. It is not intended for direct administration to humans or for therapeutic use.

Applications

CelliMax® Zeta02 plus Medium is designed for use with CHO cell lines, including CHOZN®, CHO-K1, CHO-DG44, and CHO-S, and supports cell thawing, passaging, fed-batch culture, and cryopreservation.

Storage Conditions

1. Store the powder medium sealed at 2–8 ℃, protected from light.
2. Store the prepared liquid medium sealed at 2–8 ℃, protected from light.

Shelf Life

1. The unopened powder medium has a shelf life of 2 years. Use promptly after opening. Refer to the label for details.
2. Use the liquid medium promptly after preparation.

Note: The stated shelf life is based on CelluPro's general testing methods. Users may conduct separate shelf life studies for specific applications or projects.

Preparation of Required Equipment and Materials

- ✧ Clean mixing vessel
- ✧ High purity water (HPW) such as Water for Injection (WFI) or equivalent
- ✧ Liquid mixing system with adequate mixing efficiency
- ✧ Calibrated pH meter (Recommended calibration points: 4.01, 7.00, 9.21)
- ✧ Calibrated osmometer (Recommended calibration points: 0, 300, and 700 mOsmol/kg)
- ✧ Calibrated turbidimeter (Recommended calibration points: 0.02 NTU, 20.0 NTU, 100 NTU, and 800 NTU)
- ✧ Pharmaceutical-grade sodium bicarbonate ($NaHCO_3$)
- ✧ 6 mol/L NaOH solution
  Note: Other concentrations of NaOH solution may be used with appropriate adjustment. NaOH powder is not recommended, as localized high pH may cause damage.
- ✧ Low-binding filter membranes (0.2 μm or 0.22 μm), such as polyvinylidene difluoride (PVDF), polyether sulfone (PES), or cellulose acetate (CA)

Preparation Recommendations

- ◆ Small-scale preparation (< 3 L) is not recommended to minimize variability.
- ◆ All utensils and consumables in contact with the medium should be free of mycoplasma and endotoxin contamination.
- ◆ Filtration and liquid storage systems in contact with the filtered medium should be sterile.
- ◆ Prefiltration at 0.45 μm or 0.8 μm is recommended to improve filtration efficiency and throughput.
- ◆ Dissolution time depends on preparation scale and mixing efficiency. All mixing times provided are

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reference values for small-scale preparation (e.g., < 50 L). For larger-scale preparation or systems with lower mixing efficiency, mixing times should be extended as appropriate to ensure complete dissolution and adequate mixing.

- Minimize total preparation time where possible, as prolonged preparation may increase the risk of bioburden and endotoxin contamination, provided that adequate mixing efficiency and complete powder dissolution are ensured.

Q.S. Method

- **Q.S. to final volume**: Adjust to the final volume (V_{final}). This method is recommended for small-scale preparation.
- **Q.S. to final weight**: Adjust to the final weight ($m_{final} = \rho \times V_{theoretical}$). This method is recommended for large-scale preparation.
- Recommended density for weight-based adjustment of CelliMax® Zeta02 plus Medium: **ρ = 1.008 kg/L**.
  Note: Using the preparation of 1000 L ($V_{theoretical}$) of CelliMax® Zeta02 plus Medium by weight-based adjustment as an example, the target final weight (m_{final}) is calculated as follows: $m_{final} = \rho \times V_{theoretical} = 1.008$ kg/L × 1000 L = 1008 kg. Adjust the medium solution to a final weight of 1008 kg. The resulting final volume is 1000 L.
- Prior to medium preparation, determine the appropriate Q. S. method based on the target preparation volume and the available equipment for volume adjustment (e.g., volumetric devices or weighing systems).

Preparation Method

1. Add HPW, such as Water for Injection (WFI) or equivalent, to **80–85%** of the final volume in a clean mixing vessel. Set the initial temperature to **25–30 ℃**.*
   **The preparation temperature is for reference only. Very low temperature will reduce dissolution efficiency.*
2. Start stirring. Adjust the stirring speed to ensure rapid and complete wetting of the powder **while minimizing foaming**.
3. While stirring, **slowly** add **23.06g/L*** powder medium to the vessel, avoiding formation of clumps. Mix **at least 30 minutes** until dissolved. The solution will **remain cloudy** in this step.
   The weight of all materials described herein should be controlled within a deviation of **± 1% where possible. If such deviation cannot be avoided, it should be evaluated based on actual conditions.*
4. **Slowly** add 6mol/L NaOH solution, adjust the pH to **6.00 to 6.50** until the solution is **clear**, and stir for **15 to 30** minutes*.
   **The media solution pH decreases slightly by the mixing time.*
5. **Slowly** add **2.20 g/L** sodium bicarbonate powder and continue stirring for **15–30** minutes*
   **The pH may gradually increase during mixing after dissolution of sodium bicarbonate. Mixing time should not be excessive.*
6. After stirring, test the pH of the medium solution, and **slowly** add 6mol/L NaOH solution to adjust the pH to **7.00 to 7.30**.
   Note 1: The addition amount of NaOH solution or HCl solution shall be controlled to adjust the pH, avoiding out-of-specification osmolality caused by excessive adjustment
   Note 2: If the pH falls outside the specified range during adjustment, it may be readjusted using 6 mol/L sodium hydroxide or 6 mol/L hydrochloric acid. Such adjustments may result in osmolality outside the specified range or affect the stability of medium components.
7. Add HPW (e.g., WFI or equivalent) to the final volume or target final weight* according to the determined Q.S. method. Continue stirring for **10–30** minutes.
   **Detailed procedures and precautions for adjustment (volume- or weight-based) are provided in the "Q.S. Method" section of this document.*
8. The osmolality should be **280–330 mOsmol/kg**, and turbidity should be **< 3.0 NTU**.
9. Filter sterilize using a 0.22 μm or 0.2 μm filter*.
   **It is recommended to use a low-binding filter membrane type, such as PVDF, PES or CA.*
10. Use immediately or store sealed at **2–8 ℃, protected from light**.

Preparation Before Use

- The medium contains **5.6 g/L** glucose. If additional glucose is required during culture, supplement with a 360–400 g/L glucose solution.
- The medium does not contain L-glutamine. If required, add 4–8 mM L-glutamine prior to use.
  Note: Prepare a 200 mM L-glutamine solution. The solution may be stored at 2–8 ℃ for up to one week or at -20 ℃ for up to 6 months. Avoid repeated freeze-thaw cycles.
- This medium contains 1 g/L Poloxamer 188. Additional supplementation is recommended if required.

Culture Guidelines

- Cell Culture

Table 1 Shaking incubator culture conditions

Parameter	Value
Orbital diameter	50 mm
Shaking speed	90–130 rpm (shake flask)
	200–250 rpm (shake tube)
Temperature	36.5–37 ℃
CO_2 concentration	5–8%
Humidity	80 ± 5%

- Cell Thawing

a) Rapidly thaw a vial of frozen cells in a 37 ℃ water bath (< 2 minutes).

b) Aseptically transfer the cell culture suspension to a 50 mL centrifuge tube containing 15 mL pre-warmed CelliMax® Zeta02 plus Medium.

c) Centrifuge at 115 × g for 5 minutes.

d) Aspirate and discard the supernatant.

e) Resuspend the cell pellet in pre-warmed CelliMax® Zeta02 plus Medium and transfer to a 125 mL shake flask or 50 mL shake tube. The initial seeding density should be > 0.4 × 10^6 cells/mL.

f) Incubate in a shaking incubator under the conditions described in Table 1.

g) Passage the cells at least twice after thawing at a seeding density > 0.4 × 10^6 cells/mL. Proceed to subsequent steps once the population doubling time (PDT) has stabilized.

- Cell Passaging

a) Prewarm CelliMax® Zeta02 plus Medium for 20–30 minutes.

b) Ensure a viable cell density ≥ 1.0 × 10^6 cells/mL and viability ≥ 90%, with cells maintained in the mid-exponential growth phase.

c) Based on the final culture volume, determine the appropriate volume of cell suspension to inoculate into a new shake flask or shake tube, with a recommended initial seeding density of 0.3–0.6 × 10^6 cells/mL.

d) Aseptically transfer the calculated volume of cell suspension into a shake flask or shake tube containing pre-warmed CelliMax® Zeta02 plus Medium.

e) Incubate in a shaking incubator under the conditions described in Table 1.

f) Passage cells every 2–4 days using fresh CelliMax® Zeta02 plus Medium to maintain optimal performance by following the above steps.

- Cell Adaptation

Direct Adaptation

1) CHO cells cultured in other serum-free media can be directly adapted to CelliMax® Zeta02 plus Medium. Refer to **Cell Passaging**.

2) The cells are considered adapted to CelliMax® Zeta02 plus Medium after at least two passages, with viable cell density > 2.0 × 10^6 cells/mL and viability > 90%.

Sequential Adaptation

1) For CHO cells cultured in conventional serum-containing (5–10%) or serum-free media, use a sequential adaptation strategy with a seeding density of 0.3–0.6 × 10^6 cells/mL.

2) Passage the cells into a mixture of the original culture medium and CelliMax® Zeta02 plus Medium, and gradually increase the proportion of CelliMax® Zeta02 plus Medium. The detailed sequential adaptation steps are shown in Table 2.

3) The cells are considered adapted to CelliMax® Zeta02 plus Medium after at least two passages, with viable cell density > 2.0 × 10^6 cells/mL and viability > 90%.

Table 2 Sequential adaptation scheme

Ratio (Original: CelliMax® Zeta02 plus Medium) (%)	Seeding density (× 10^6 cells/mL)	Acceptance criterion to proceed to next adaptation step
75:25	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%
50:50	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%
25:75	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%
0:100	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%

- Cell Cryopreservation

a) Harvest cells in mid-exponential growth phase with viability > 90%.

b) Determine the viable cell density and calculate the volume of cryopreservation medium required to achieve a final cell density of ≥ 1.0 × 10^7 cells/mL.

c) Prepare cryopreservation medium consisting of 90% CelliMax® Zeta02 plus Medium and 10% dimethyl sulfoxide (DMSO). Store at 2–8 ℃ prior to use.

d) Centrifuge at 115 × g for 5 minutes, then resuspend the cell pellet in pre-equilibrated cryopreservation medium.

e) Transfer the cell suspension into sterile cryovials.

f) Freeze using a controlled-rate freezing method (approximately -1 ℃/min) according to standard procedures.

g) Transfer frozen cells to liquid nitrogen (-196 ℃) for long-term storage.

Ordering Information

Table 3 Ordering information

Product	Order Reference No.	Format
CelliMax® Zeta02 plus Medium	CPDP089-10L	Powder
	CPDP089-100L	
	CPDP089-500L	

**For more information, please visit www.cellupro.com or contact sales@cellupro.com.*

**Specifications are subject to change without prior notice, except for process changes.*

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