CelliMax® CHO FGa Medium

Instructions for Use

Cat. No.: **CPDP062 (Powder)**

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Product Description

CelliMax® CHO FGa Medium is a chemically defined (CD) feed medium. It is free of animal-derived components and hydrolysates, and does not contain L-glutamine, proteins, growth factors, or phenol red. When used in combination with CelliMax® CHO FGb Medium, 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® CHO FGa Medium is designed for use with CHO cell lines, including CHO-K1, CHO-DG44, CHO-S, and CHO-DXB11.

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)
- ✧ 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)
- ✧ 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 (< 1 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 reference values for small-scale preparation (e.g., < 50 L). For larger-scale preparation or systems with

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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® CHO FGa Medium: **ρ = 1.062 kg/L**.
  Note: Using the preparation of 1000 L ($V_{theoretical}$) of CelliMax® CHO FGa Medium by weight-based adjustment as an example, the target final weight (m_{final}) is calculated as follows: $m_{final} = \rho \times V_{theoretical} = 1.062$ kg/L × 1000 L = 1062 kg. Adjust the medium solution to a final weight of 1062 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 **70–75%** 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*. S**lowly** add **164.50 g/L*** powder medium to the vessel, avoiding formation of clumps. Mix **at least 30 minutes** until dissolved. The solution may **appear slightly turbid** at this stage.
   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.*
   **During preparation, to avoid excessive foaming, maintain a moderate stirring speed while ensuring efficient dissolution.*
   **During the initial stage of powder addition, accumulation above the liquid surface is normal. Maintain normal stirring speed, and the powder will gradually dissolve.*
3. **Slowly** add 6 mol/L NaOH solution to adjust the pH to **6.70–6.90** until the solution becomes **clear**, then continue stirring for **15–30** minutes*.
   **The pH of the medium may decrease slightly over time during mixing.*
4. After stirring, measure the pH of the medium. If the pH is below **6.70**, **slowly** add 6 mol/L NaOH solution to adjust the pH to **6.70–6.90**.
   Note 1: The amount of NaOH solution added should be carefully controlled to avoid excessive adjustment, which may result in osmolality outside the specified range.
   Note 2: If the pH falls outside the specified range during adjustment, it may be readjusted using 6 mol/L sodium hydroxide solution or 6 mol/L hydrochloric acid solution. Such adjustments may result in osmolality outside the specified range or affect the stability of medium components.
5. 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 **10–15** minutes.
   Note: Detailed procedures and precautions for adjustment (volume- or weight-based) are provided in the "Q.S. Method" section of this document.
6. The osmolality should be **260–300 mOsmol/kg** (tested at 5-fold dilution), and turbidity should be **< 3.0 NTU**.
7. 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.*
8. Use immediately or store sealed at **2–8 ℃, protected from light**.

Preparation Before Use

- The medium contains **60 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 10–30 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 does not contain growth factors. If necessary, it is recommended to supplement with growth factors at μg levels to support normal cell growth.
- 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%

- Fed-batch Culture Strategy

a) Inoculate the adapted cells into CelliMax® basal medium portfolio at a viable cell density of 0.4–0.7 × 10^6 cells/mL with viability of > 95%.

b) When the viable cell density reaches ≥ 3.0 × 10^6 cells/mL, perform the first feeding.

c) Add CelliMax® CHO FGa Medium at 3–5% and CelliMax® CHO FGb Medium at 0.3–0.5% based on the initial culture volume. Feed every other day. Monitor glucose concentration daily to maintain residual glucose no less than 1 g/L in the culture supernatant. If glucose supplied by CelliMax® CHO FGa Medium is insufficient to meet cellular demand, supplement with glucose solution (360–400 g/L). Refer to Table 2 for details of the feeding strategy.

Table 2 Recommended fed-batch culture strategy

Product Name	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
CelliMax® CHO FGa Medium	3–5%		3–5%		3–5%		3–5%		3–5%		3–5%
CelliMax® CHO FGb Medium	0.3–0.5%		0.3–0.5%		0.3–0.5%		0.3–0.5%		0.3–0.5%		0.3–0.5%
Residual glucose in culture supernatant	> 1 g/L										

Note 1: The recommended ratio of CelliMax® CHO FGa Medium to CelliMax® CHO FGb Medium is 10:1.
Note 2: This feeding strategy is recommended for a peak cell density of 10–30 × 10^6 cells/mL. If the cell density is outside this range, increase or decrease the feeding percentage accordingly.
Note 3: The fed-batch culture strategy can be appropriately adjusted based on inoculation density, process parameters, and temperature shift conditions.

d) Terminate the culture when a pre-defined end criterion is reached, e.g., on D14–D16 post-inoculation or when cell viability is < 70%.

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Ordering Information

Table 3 Ordering information

Product	Order Reference No.	Format
CelliMax® CHO FGa Medium	CPDP062-1L	Powder
	CPDP062-5L	
	CPDP062-20L	

**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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