DurePro® HD Perfusion Medium

Instructions for Use

Cat. No.: CPDP082 (Powder)

Product Description

DurePro® HD Perfusion Medium is a chemically defined (CD) perfusion medium. It is free of animal-derived components and hydrolysates, and does not contain L-glutamine, proteins, growth factors, or phenol red. DurePro® HD Perfusion Medium is a ready-to-use, high-nutrient perfusion medium specifically designed for continuous protein production during the N-stage. It enhances specific productivity and supports long-term steady-state maintenance of CHO cells.

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

DurePro® HD Perfusion Medium is suitable for a variety of CHO cell lines, including CHO-K1, CHO-DG44, CHO-S, and DUBX-11.

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 reference values for small-scale preparation (e.g., <

CelluPro, Ltd.
60 Beijing Middle Road, Economic & Technological Development Area, Yantai, Shandong, China
Phone：+86 535 3573438
E-mail：support@cellupro.com
www.cellupro.com

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 DurePro® HD Perfusion Medium: **ρ = 1.015 kg/L**.
  Note: Using the preparation of 1000 L ($V_{theoretical}$) of DurePro® HD Perfusion Medium by weight-based adjustment as an example, the target final weight (m_{final}) is calculated as follows: $m_{final} = \rho \times V_{theoretical} = 1.015$ kg/L × 1000 L = 1015 kg. Adjust the medium solution to a final weight of 1015 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. Fill a clean mixing vessel to **85–95%** of the final volume with HPW (e.g., WFI or equivalent) at **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 **35.45 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.*
4. **Slowly** add 6 mol/L NaOH solution to adjust pH to **6.6–6.8** and continue stirring for **15–30** 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, measure the pH of the medium. **Slowly** add **6 mol/L** NaOH solution to adjust pH to **7.00–7.30**.
   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 **310~370mOsmol/kg**, and turbidity should be **< 3.0 NTU**.
9. Filter sterilize using a 0.2 μm or 0.22 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 **10 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 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 2 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	200–300 rpm (shake tube)
Culture volume	10–15 mL
Temperature	36.5–37 ℃
CO_2 concentration	5–8%
Humidity	80 ± 5%

- Shake Tube Semi-perfusion Culture

a) Cells should be seeded in 50 mL TPP tubes with a culture volume of 10 mL at a seeding density of 0.5–1.0 × 10^6 cells/mL, with a shaking speed of 200–225 rpm.

b) When viable cell density reaches 3.0–5.0 × 10^6 cells/mL, perform daily centrifugation medium replacement with an equal volume of fresh CelliMax® series basal medium. Increase the shaking speed to 250 rpm.

c) When viable cell density ≥ 15.0 × 10^6 cells/mL, perform daily centrifugation medium replacement using DurePro® HD Perfusion Medium. Further increase the shaking speed to 300 rpm.

d) When cell density no longer increases under the current perfusion conditions, the culture is considered to have reached the steady state. It is recommended to maintain cell density at 90–95% of the steady state by bleeding.

e) Perform daily bleeding and centrifugation, and replace the medium with fresh DurePro® HD Perfusion Medium. Take samples of the cell culture supernatant every 2–3 days for titer determination.

f) Continue the semi-perfusion culture until the cell viability drops below 80%, at which point the culture can be terminated.

Note 1: Shake tube semi-perfusion culture is performed by centrifugation and medium replacement every 24 or 12 hours, corresponding to a perfusion volume of 1–2 VVD per day.

Note 2: During shake tube semi-perfusion culture, glucose consumption during semi-perfusion is significantly higher than in conventional fed-batch processes. Glucose levels should be monitored daily. If the glucose content in the perfusion medium cannot meet daily consumption requirements, additional glucose solution should be supplemented. It is recommended to maintain residual glucose levels at no less than 2–3 g/L.

Ordering Information

Table 3 Ordering information

Product	Order Reference No.	Format
DurePro® HD Perfusion Medium	CPDP082-5L	Powder
	CPDP082-50L	
	CPDP082-500L	

**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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CelluPro, Ltd.
60 Beijing Middle Road, Economic & Technological Development Area, Yantai, Shandong, China

Phone：+86 535 3573438
E-mail：support@cellupro.com
www.cellupro.com