

## Chemically Defined CHO Cell Feed Medium

### A. Product Description

#### Components

HelixCHO Feed is a chemically defined feed medium formulated for CHO cell cultures. It is hydrolysate-free, animal-origin free (AOF), and does not contain L-glutamine. It contains 84 g/L D-glucose.

#### Application

- Designed for high-density fed-batch processes across multiple Chinese hamster ovary (CHO) cell subtypes.
- Recommended cell subtypes: CHO-K1(Merck), CHOZN, CHO DG44.
- Recommended companion media: StarCHO™, VegaCHO™, HelixCHO™ and CDFS36™ Feed Supplement.
- This product is intended for research use or further manufacturing, including large-scale biopharmaceutical production. It is not intended for direct human administration or use as a drug substance or drug product.

#### Storage and Shipping conditions

Storage: 2-8°C, dry and protected from light.

Shipping conditions: insulated foam container with ice packs.

#### Shelf Life

Liquid: 12 months | Powder: 24 months

### B. Dry Powder Reconstitution Method

1. Use a clean container.
2. Measure out 80% of the final volume of ultrapure water or WFI (Water for Injection) with temperature at 25°C to 35°C.
3. Add 180.09 g/L of HelixCHO Feed Dry Powder Medium (DPM) slowly to the water. Keep stirring for 20 minutes.
4. Add 5 N NaOH slowly to increase pH to 6.90-7.10. Keep stirring for 30 minutes. The solution will become clear. Measure the pH and adjust as needed to 6.90-7.10 with 5 N NaOH or 5 N HCl.

5. Adjust to the final volume with ultrapure water or WFI and continue stirring for 10 minutes. Measure the final pH and osmolality and ensure the final pH is 6.90-7.10.
6. Sterilize immediately by filtration through a low-protein binding membrane, such as polyethersulfone (PES), with a pore size of 0.20-0.22 µm.
7. Label and store the reconstituted medium at 2°C to 8°C, protected from light.

### C. Volume Adjustment

1. **Volumetric adjustment:** During medium preparation, adjust the solution to the target volume based on the actual volume ( $V_{\text{actual}}$ ). This method is recommended for small-scale preparations.
2. **Gravimetric adjustment:** During medium preparation, adjust the solution by weight ( $m = \rho \times V_{\text{actual}}$ ). This method is recommended for large-scale preparations. Recommended density for HelixCHO Feed:  $\rho = 1.062 \text{ g/cm}^3$ .

### D. Culture Conditions

- Temperature: 37°C
- Humidity: 80%
- CO<sub>2</sub> concentration: 5-8%
- Shaker settings:
  - 120 rpm with a 50 mm orbital diameter
  - 170 rpm with a 25 mm orbital diameter

### E. Recommended Protocol for Fed-Batch Process

1. The seed cell density should be between  $3 \times 10^6$  and  $6 \times 10^6$  cells/mL, with viability above 95% and a stable population doubling time (PDT).
2. Day 0: Inoculate cells at a viable cell density of  $1.0 \times 10^6$  cells/mL into OPM basal medium.
3. When the viable cell density reaches  $\geq 3.5 \times 10^6$  cells/mL (typically by day 2), perform the first feeding.

4. Addition of HelixCHO Feed is recommended between 3-6% of the initial culture volume, with feeding every other day after feeding is initiated. For best results, HelixCHO Feed should be paired with an appropriate OPM basal medium and OPM feed supplement based on the OPM Medium & Feed Selection Guide as below: <http://opmbio.com/media-selection-guide-na>
5. Glucose should be monitored and adjusted daily. A suggested feeding strategy for HelixCHO Feed is displayed in Table 1.

Table 1. Suggested feeding strategy for HelixCHO Feed

Culture Day	2	4	6	8	10	12	14	16
HelixCHO Feed	5%	5%	6%	6%	6%	6%	5%	Harvest
OPM Feed Supplement	0.5%	0.5%	0.6%	0.6%	0.6%	0.6%	0.5%	Harvest

*\* Optimization of feed volumes and timing of feedings should be based on actual process performance and requirements. Other process parameters such as temperature downshift should be followed based on platform development or clone performance.*

## F. Ordering Information

Product Name	SKU	Volume
HelixCHO Feed Medium	P253322-01	1000 mL
HelixCHO Feed DPM	P253590-01	1 L
	P253590-02	10 L
	P253590-03	50 L
	P253590-04	100 L