HelixCHO™

Chemically Defined CHO Cell Culture Medium

A. Product Description

Components

HelixCHO is a chemically defined basal medium formulated for CHO cell cultures. It is hydrolysate-free, animal-origin free (AOF), and does not contain L-glutamine. It contains 6 g/L D-glucose.

Application

- Designed for thawing, passaging, and supporting high-density fed-batch processes across multiple Chinese hamster ovary (CHO) cell subtypes.
- Recommended cell subtypes: CHOZN CHO-K1(Merck), etc.
- Recommended companion feeds: HelixCHO™ Feed 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. It is recommended that the one-time reconstitution volume should not be less than 1 L.
2. Measure out 90% of the final volume of ultrapure water or water for injection (WFI) with temperature between 25°C and 35°C.
3. Add 21.73 g/L of HelixCHO Dry Powder Medium (DPM) slowly to the water. Keep stirring for 10 minutes.
4. Add 2.22 g/L Sodium Bicarbonate to the solution. Keep stirring for 10 minutes.
5. Add 5 N NaOH slowly to increase the pH to 8.30-8.50. Keep stirring for 30 minutes. The solution will become clear.
6. Adjust pH to 6.90-7.10 by slow addition of 5 N HCl.
7. Adjust to the final volume with ultrapure water or WFI and keep stirring for 5 minutes.
8. Adjust pH to 6.90-7.10 with 5 N NaOH or 5 N HCl.
9. Adjust the osmolality to 290 ± 15 mOsm/kg by adding NaCl using the following formula:

$$NaCl\ Powder\ W\ (g) = \frac{V_T \times (290 - MV_{Osm})}{31.5}$$

V_T: Target volume
MV_{OSM}: Measured value of osmolality

10. Keep stirring for an additional 10 minutes. Sterilize immediately by filtration through a low-protein binding membrane, such as polyethersulfone (PES), with a pore size of 0.20-0.22 μm.
11. Label and store the reconstituted medium at 2-8°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_{actual}). This method is recommended for small-scale preparations.
2. **Gravimetric adjustment:** During medium preparation, adjust the solution by weight ($m = \rho \times V_{actual}$). This method is recommended for large-scale preparations. Recommended density for HelixCHO Medium: $\rho = 1.007\ g/cm^3$.

D. Culture Conditions

- Temperature: 37°C
- Humidity: 80%
- CO_2 concentration: 5–8%
- Shaker settings:
  - 120 rpm with a 50 mm orbital diameter
  - 170 rpm with a 25 mm orbital diameter

E. Cell Thawing

1. Rapidly thaw frozen cells in a 37°C water bath within 2 minutes.

2. Transfer the entire contents of the cryovial into a 125 mL shake flask containing 30 mL of HelixCHO Medium.

3. Incubate the flask in a shaking incubator under the recommended culture conditions.

4. Passage the cells at least twice until they are fully recovered and the population doubling time (PDT) stabilizes before proceeding to subsequent operations.

F. Cell Passaging

1. Cells cultured in serum-free medium can be directly inoculated into HelixCHO Medium without centrifugation. The seeding density should follow the steps below and be adjusted as needed.

2. Use cells in early- to mid-logarithmic growth phase with a viable cell density (VCD) ≥ 1×10^6 cells/mL and viability ≥ 90%. Calculate the required inoculum volume based on the target seeding density (0.5×10^6 – 1.0×10^6 cells/mL) and final culture volume using the following formula:

$$V_{Seed} = \frac{VCD_{Target} \times V_{Culture}}{VCD_{Current}}$$

VCD_{Target}: Desired seeding density for the next passage
$VCD_{Current}$: Viable cell density of the culture before passaging

3. Aseptically transfer the calculated volume of seed culture into a shake flask containing the required volume of HelixCHO Medium.

4. Place the flask in a shaking incubator and continue culturing under standard conditions.

5. Passage the culture every 2–3 days using fresh medium following the same procedure.

G. Cell Cryopreservation

1. Prepare cells in early- to mid-logarithmic phase with viability > 90% and good morphology.

2. Determine viable cell density and calculate the required volume of cell freezing medium. The final freezing density should be > 1×10^7 cells/mL.

3. Prepare fresh cell freezing medium: 90% HelixCHO Medium + 10% dimethylsulfoxide (DMSO).

4. Collect cells by centrifugation at 300 × *g* for 5 minutes.

5. Carefully aspirate or decant the supernatant, resuspend the cell pellet in freezing medium and aliquot into cryovials of appropriate size.

6. Place cryovials in a controlled-rate freezing container, incubate at 2–8°C for 10–15 minutes, then freeze overnight at -80°C before transferring to liquid nitrogen for long-term storage.

H. Ordering Information

Product Name	SKU	Volume
HelixCHO Medium	P254078-01	1000 mL
HelixCHO DPM	P258076-01	1 L
	P258076-02	10 L
	P258076-03	50 L
	P258076-04	100 L
	P258076-05	500 L