

Chemically Defined CHO Cell Culture Medium

A. Product Description

Components

VegaCHO 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: CHO DG44, etc.
- Recommended companion feeds: VegaCHO™ Feed Plus, HelixCHO™ Feed, VectorCHO™ 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

- Use a clean container. It is recommended that the one-time reconstitution volume should not be less than 1 L.
- Measure out 90% of the final volume of ultrapure water or water for injection (WFI) with temperature between 25°C and 35°C.
- Add 21 g/L of VegaCHO Dry Powder Medium (DPM) slowly to the water. Keep stirring for 10 minutes.

- Add 2.22 g/L Sodium Bicarbonate to the solution. Keep stirring for 10 minutes.
- Add 5 N NaOH slowly to increase the pH to 8.30-8.50. Keep stirring for 30 minutes. The solution will become clear.
- Adjust pH to 6.90-7.10 by slow addition of 5 N HCl.
- Adjust to the final volume with ultrapure water or WFI and keep stirring for 5 minutes.
- Adjust pH to 6.90-7.10 with 5 N NaOH or 5 N HCl.
- Adjust the osmolality to 290 ± 15 mOsm/kg by adding NaCl using the following formula:

$$\text{NaCl Powder } W(g) = \frac{V_T \times (290 - MV_{osm})}{37.5}$$

V_T : Target volume

MV_{osm} : Measured value of osmolality

- 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 .
- Label and store the reconstituted medium at 2-8°C, protected from light.

C. Volume Adjustment

- 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.
- 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 VegaCHO Medium: $\rho = 1.009 \text{ g/cm}^3$.

D. Culture Conditions

- Temperature: 37°C
- Humidity: 80%
- CO₂ concentration: 5–8%
- Shaker settings:
 - 120 rpm with a 50 mm orbital diameter
 - 170 rpm with a 25 mm orbital diameter

E. Cell Thawing

- Rapidly thaw frozen cells in a 37°C water bath within 2 minutes.
- Transfer the entire contents of the cryovial into a 125 mL shake flask containing 30 mL of VegaCHO Medium.
- Incubate the flask in a shaking incubator under the recommended culture conditions.
- 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

- Cells cultured in serum-free medium can be directly inoculated into VegaCHO Medium without centrifugation. The seeding density should follow the steps below and be adjusted as needed.
- Use cells in early- to mid-logarithmic growth phase with a viable cell density $\geq 1 \times 10^6$ cells/mL and viability $\geq 90\%$. 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:

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

VCD_{Target} : Desired seeding density for the next passage

VCD_{Current} : Viable cell density of the culture before passaging

- Aseptically transfer the calculated volume of seed culture into a shake flask containing the required volume of VegaCHO Medium.
- Place the flask in a shaking incubator and continue culturing under standard conditions.
- Passage the culture every 2–3 days using fresh medium following the same procedure.

G. Cell Cryopreservation

- Prepare cells in early- to mid-logarithmic phase with viability $> 90\%$ and good morphology.
- Determine viable cell density and calculate the required volume of cell freezing medium. The final freezing density should be $> 1 \times 10^7$ cells/mL.
- Prepare fresh cell freezing medium: 90% VegaCHO Medium + 10% dimethylsulfoxide (DMSO).
- Collect cells by centrifugation at $300 \times g$ for 5 minutes.
- Carefully aspirate or decant the supernatant, resuspend the cell pellet in freezing medium and aliquot into cryovials of appropriate size.
- 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
VegaCHO Medium	P121662-01	1000 mL
	P106390-01	1 L
	P106390-02	10 L
VegaCHO DPM	P106390-03	50 L
	P106390-04	100 L
	P106390-05	500 L