

VegaCHO™

Chemically Defined Fed Batch Medium

— For Biomanufacturing



VegaCHO™ is a chemically-defined medium designed for high density suspension culture of Chinese Hamster Ovary (CHO) cell lines. It is free of any animal-origin components, and contains no hydrolysates, proteins or components of unknown composition. This medium supports high level expression of recombinant proteins and therapeutic antibodies. In conjunction with OPM's next generation high performance feeds VegaCHO™ Feed or AltairCHO™ Feed, higher growth & viability, and higher expression level of the target molecule can be achieved.

Application

VegaCHO™ cell culture medium can be used to reach & maintain high density suspension culture and to achieve high titer production in fed-batch applications. This medium is intended for large scale manufacturing of therapeutic biomolecules, as well as for research purposes, but not for human or any therapeutic use.

Storage & Transportation

Store at 2~8°C, dark and dry

Ship at Room temperature (Liquid), Blue ice (Dry powder)

Shelf Life

VegaCHO™ Medium Liquid: 12 months

VegaCHO™ Dry Powder: 24 months

Reconstitution Method for Dry Powder

(Without Glutamine and NaHCO₃)

- 1.Fill a clean mixing vessel to 90% of the final volume with high quality purified water, such as WFI at ambient temperature (25°C to 35°C). Start mixing. For example, to prepare 1 liter of growth medium, start with 900 mL of water.
- 2.Add VegaCHO™ DPM at 21.0 g/L slowly to the vessel, avoiding formation of clumps. Keep stirring for 10 minutes.
- 3.Add 2.22 g/L NaHCO₃ to the vessel and keep stirring.
- 4.Add 5N NaOH slowly to increase pH to 8.3-8.5. Keep stirring for 30 minutes. Solution will be clear.
- 5.Adjust pH to 7.0 with 5N HCl slowly.
- 6.Adjust to the final volume with high quality purified water, such as WFI and keep stirring 5 minutes
- 7.Adjust pH to 7.0 use 5N NaOH or 5N HCl.
- 8.Adjust osmolality to 290 ± 15 mOsm/kg with calculated amount of NaCl. Calculation formula: NaCl powder $W(g) = VT \times (290 - MVOsm) / 31.5$, VT: Target volume, MVOsm: measured value of Osm.
- 9.Mix for an additional 10 minutes.
- 10.Sterilize immediately by membrane filtration.
- 11.Label as "VegaCHO Medium".
- 12.Store media at 2°C to 8°C with protection from light.

Quality Specifications

Specifications	VegaCHO™ Medium	VegaCHO™ DPM
Appearance	Red clear liquid	Off -white or light yellow powder
pH	7.0~7.5	7.0~7.5
Osmolality (mOsm/kg)	270~300	270~300
Solubility	---	Good by following the reconstitution instructions
Endotoxin (EU/mL)	<1.0	<1.0
Sterility test	Negative	---

Cell Culture Conditions

37°C, 80% humidity, 5~8%CO₂

Shaker speed 110-150 rpm (amplitude: 50mm).

Cell Recovery

1. Rapidly thaw (<2 min) a vial of frozen cells in a 37 °C water bath.
2. Transfer the entire contents aseptically into a 125 mL shake flask containing 30 mL prewarmed VegaCHO™ cell culture medium.
3. Incubate at 37 °C in a humidified atmosphere of 5%~8% CO₂ in air on a shaker (rotating at 110~130 rpm (amplitude: 50mm).
4. Passage the cells for at least twice until fully recovered. Proceed according normal procedure after the Population Doubling Time stays stable.

Cell Culture Passaging

1. Prewarm VegaCHO™ cell culture medium at 37 °C for 20~30min.
2. Proceed if VCD $\geq 1 \times 10^6$ /mL & viability $\geq 90\%$. Cultures should be passaged during the mid-log phase.
3. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 0.5×10^6 cells/mL in prewarmed VegaCHO™ cell culture medium
4. Incubate flasks in a humidified 37 °C incubator with 5%~8% CO₂ on an orbital shaker at 110~150rpm (amplitude: 50mm).
5. Passage cells by repeating the above steps every 2~4 days.

Medium Adaptation

Direct Medium Adaptation

1. Cell lines may be adapted directly from serum-free media into VegaCHO™ cell culture medium. The seeding cell density can be referred to the passaging instructions or should be determined individually.
2. Cells should be passaged for a few times.
3. Adaptation is completed when the cultures attain stable VCD of 2×10^6 /mL and viability $\geq 90\%$ within 3~4 days over at least 2~3passages.

Sequential Medium Adaptation

1. For certain cell lines cultured in serum-free media, or in presence of 5~10% serum, sequential adaptation method is recommended.
2. Monitor the cell growth until the cell density has reached $\geq 2 \times 10^6$ cells/mL.
3. Dilute the cells with a ratio of 25:75 (VegaCHO™ vs current medium), and then further dilute the culture until the cells grow well under this condition. Increase the proportion of VegaCHO™ in each subsequent operation, as is shown in the table.

- Adaptation is completed when the cultures in 100% VegaCHO™ Cell culture medium attain stable VCD of 2×10^6 /mL and viability $\geq 85\%$ within 3~4 days over at least 2~3 passages.

VegaCHO™: current medium (%)	Seeding density ($\times 10^5$ cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
25 : 75	3 ~ 4	VCD & Viability	VCD $\geq 2 \times 10^6$ /mL, Viability $\geq 90\%$ over 2 passages
50 : 50	3 ~ 4	VCD & Viability	VCD $\geq 2 \times 10^6$ /mL, Viability $\geq 90\%$ over 2 passages
75 : 25	3 ~ 4	VCD & Viability	VCD $\geq 2 \times 10^6$ /mL, Viability $\geq 90\%$ over 2 passages
90 : 10	3 ~ 4	VCD & Viability	VCD $\geq 2 \times 10^6$ /mL, Viability $\geq 90\%$ over 2 passages
100 : 0	3 ~ 4	VCD & Viability	VCD $\geq 2 \times 10^6$ /mL, Viability $\geq 90\%$ over 2 passages

Cryopreservation

- Harvest the desired quantity of cells in mid-log phase of growth with viability over 90%.
- Determine VCD to ensure that the final cell density is $> 1 \times 10^7$ /ml.
- Prepare the freezing medium consisting of 90% VegaCHO™ Cell culture medium and 10% dimethyl sulfoxide (DMSO). Let the freezing medium cool down to 4°C.
- Harvest cells by centrifugation at 400xg for 5 minutes. Remove the supernatant and resuspend the cell pellet with the cold freezing medium at $> 1 \times 10^7$ /ml.
- Transfer the suspension to sterile cryo-vials.
- Place the vials in a cryo-box or a controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- For long-term storage, transfer the vials to liquid nitrogen.

Order Information

Cell Culture Media

Name	Cat No.	Type	Volume
VegaCHO™ Medium	P121662	Liquid	1000mL
VegaCHO™ DPM	P106390	Dry powder	10L / 50L / 100L

High Performance Feeds

Name	Cat No.	Type	Volume
VegaCHO™ Feed	P134305	Liquid	500mL
VegaCHO™ Feed DPM	P120826	Dry powder	10L / 50L
AltairCHO™ Feed	C675219	Liquid	500mL
AltairCHO™ Feed DPM	C679332	Dry powder	10L / 50L

Highly Concentrated Feeds

Name	Cat No.	Type	Volume
CDFS36	C217836	Liquid	500ml / 1000ml
CDFS36 DPM	C672069	Dry powder	1L / 2L / 5L / 10L / 50L / 100L

Cell Culture Supplements

Name	Cat No.	Type	Volume
OPM GAL+V2 Galatosylation enhancer	S81912	Liquid	100mL / 1000mL
OPM-ACA Anti-clumping agent	S0907001	Liquid	100mL / 500mL / 1000mL

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