



Optimization
Makes Differences

OPM-CHO CDP9

Chemically Defined Fed Batch Cell Culture Medium

— For Biomanufacturing



OPM-CHO CDP9 is a chemically-defined cell culture medium designed for high density suspension culture of Chinese Hamster Ovary (CHO) cell lines (e.g. CHO-K1, CHO-DG44, CHO-S). It is free of any animal-origin components, and contains no hydrolysates, proteins, growth factors or components of unknown composition. This medium supports high level expression of recombinant proteins and therapeutic antibodies. In conjunction with OPM’s high performance feeds and highly concentrated feeds, higher growth & viability, and higher expression level of the target molecule can be achieved.

Application

OPM-CHO CDP9 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

OPM-CHO CDP9 Medium Liquid: 12 months
OPM-CHO CDP9 Dry Powder: 24 months

Reconstitution Method for Dry Powder

1. Measure out 90% of final required volume of purified water intended for cell culture use, e.g. WFI. Recommended water temperature is 25~35°C (minimum final volume \geq 1L).
2. Slowly add dry powder medium at 18.87 g/L while stirring, and continue mixing for 10 minutes. Residual powder attached to the vessel wall should be taken into the solution.
3. Add 2.22 g/L NaHCO₃ to the solution, continue to stir.
4. Adjust PH to 8.3~8.5 with 5N NaOH and stir for 20~30 minutes until completely dissolved.
5. Adjust PH to 7.0 by slowly adding 5N HCl.
6. Add cell culture grade purified water to 100% final volume. Continue to stir for 5 minutes. Adjust PH to 7.0 using 5N HCl or 5N NaOH.
7. Adjust osmolality to 285±10 mOsm/kg using NaCl (Calculate osmolality: $\text{required NaCl(g)} = \text{final solution volume(L)} \times (285 - \text{measured value}) / 31.5$).
8. Continue to stir for 10 minutes. Sterile filter using a membrane filter with a pore size of 0.22 micron.

Quality Specifications

| Specifications | OPM-CHO CDP9 Medium | OPM-CHO CDP9 DPM |
|----------------------|---------------------|---|
| Appearance | --- | Off-white or light yellow powder |
| pH | 7.0~7.4 | 7.0~7.4 |
| 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 OPM-CHO CDP9 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 recovery. Proceed according normal procedure after the Population Doubling Time stays stable.

Cell Culture Passaging

1. Prewarm OPM-CHO CDP9 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 OPM-CHO CDP9 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 2~3 days.

Medium Adaptation

Direct Medium Adaptation

1. Cell lines may be adapted directly from serum-free media into OPM-CHO CDP9 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 2~3 passages.

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 (OPM-CHO CDP9 vs current medium), and then further dilute the culture until the cells grow well under this condition. Increase the proportion of OPM-CHO CDP9 in each subsequent operation, as is shown in the table.
4. Adaptation is completed when the cultures in 100% OPM-CHO CDP9 Cell culture medium attain stable VCD of 2×10^6 /mL and viability $\geq 90\%$ within 3~4 days over at least 2~3 passages.

| OPM-CHO CDP9: 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

1. Harvest the desired quantity of cells in mid-log phase of growth with viability over 90%.
2. Determine VCD to ensure that the final cell density is $> 1 \times 10^7$ /ml.
3. Prepare the freezing medium consisting of 90% OPM-CHO CDP9 Cell culture medium and 10% dimethyl sulfoxide (DMSO). Let the freezing medium cool down to 4°C.
4. 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.
5. Transfer the suspension to sterile cryo-vials.
6. Place the vials in a cryo-box or a controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. For long-term storage, transfer the vials to liquid nitrogen.

Order Information

Cell Culture Media

| Name | Cat No. | Type | Volume |
|---------------------|-------------|------------|--------|
| OPM-CHO CDP9 Medium | P081909-001 | Liquid | 1000ml |
| OPM-CHO CDP9 DPM | P091909-010 | Dry powder | 10L |
| | P091909-050 | Dry powder | 50L |

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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