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Pharma & Biopharma Raw Material Solutions

Cellvento® CHO-220

Chemically defined cell culture medium for fed-batch applications

Mammalian cell expression systems are the dominant tool today for producing complex biotherapeutic proteins. Different CHO hosts are routinely used to develop such biologics. One extensively used cell line is CHO-K1, which can be paired with several expression systems like UCOE or the glutamine synthetase (GS) system.

The media and feed system described in this Process Guidance was specifically developed to grow CHO-K1 cells. The production medium supports initial cell growth and production while the feed or supplement(s) are added to replenish depleted nutrients required for cellular function and to maintain and extend the production phase of the culture in fed-batch mode.

As the performance of production media and their companion feed(s) is typically interdependent, optimizing a feeding strategy is a crucial step to achieve high cellular growth while maintaining high specific productivity. This document provides the basis for initiating feed optimization activities, but fine-tuning an effective feeding strategy should be considered.

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The fed-batch media system

Cellvento® CHO-220 medium and its companion Cellvento® Feed-220 are chemically defined, non-animal origin products designed for use with CHO cell-based mammalian cell culture. The medium and its feeds are effective at achieving and supporting high-density cell growth and competitive productivity with CHO-K1 suspension cell types and expression systems, but may also be appropriate for use with other CHO cell lines. As with all fed-batch processes, however, optimization of feeding volumes and feed frequency is recommended.

Production medium and main feed

1.02577.0010 Cellvento® CHO-220 medium

1.02578.0003 Cellvento® Feed-220

Additional feed supplements

1.02452.0025 L-Cysteine for cell culture media

1.02413.0100 L-Tyrosine di-sodium salt dihydrate

Additives

1.00286.1000 L-Glutamine EMPROVE® exp

1.37013.2500 Sodium hydrogen carbonate

Filtration

GPWP02500 Millipore Express® PLUS Membrane, 0.22 µm, 25 mm

GVWP02500 Durapore® Membrane 0.22 µm, 25 mm

All components are available individually.

Applications

- Cellvento® CHO-220 medium and its companion feeds have been designed for use with recombinant CHO-K1 suspension cells, but may also be suitable for other CHO cell lines.
- Cellvento® CHO-220 medium should be used as an amplification and production medium in fed-batch applications (together with its companion feed product Cellvento® Feed-220).
- Cellvento® products are designed to allow and provide for flexibility in feed and feed supplement optimization of fed-batch processes.

Using Cellvento® CHO-220 medium in fed-batch mode

- Add 4–8 mM L-glutamine to Cellvento® CHO-220 medium prior to use with non-GS CHO cell lines.
- Cellvento® Feed-220 does not require any additional supplementation with L-glutamine for use in fed-batch culture.
- Optimal volumes and timing of Cellvento® Feed-220 and Cys/Tyr feed administration should be determined experimentally (see point 5 in this Process Guidance).
- Glucose should be monitored daily and added separately during feeding to maintain appropriate levels throughout the fed-batch culture.
- Cell selection agents should be added as required during the seed train. In general, we recommend removing the selective pressure during the fed-batch production step and culture.

Options for Cellvento® CHO-220 media system evaluation

1. Direct media adaptation

Cell lines may be adapted directly into Cellvento® CHO-220 medium. Cells should be seeded at 3×10^5–5×10^5 cells/mL, then sub-cultured when densities reach 1×10^6–3×10^6 cells/mL and ≥80% viability. Adaptation is complete when cells attain a stable doubling time (20–30 hours) and viability is ≥90% over at least 2–3 passages.

2. Sequential media adaptation

The adaptation guidance provided below relies on regular sub-culturing of cells to maintain cultures in a logarithmic growth phase. This typically means that cells should be passaged every 3 to 4 days. At least 2 passages at each adaptation step are recommended to ensure that cells appropriately adjust to their new media environments.

Ratio of current media vs. Cellvento® CHO-220 medium (in %)	Seeding density (x 10^5 cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
75:25	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viability >80% over at least 2 passages
50:50	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viability >80% over at least 2 passages
25:75	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viability >80% over at least 2 passages
10:90	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viability >80% over at least 2 passages
0:100	3.0	Cell density, viability in mid-log growth phase	Adaptation complete when cells maintain normal doubling time; Viability ≥90% over at least 2 passages

3. Cryopreservation

Viable cell banks may be created by freezing cells in 90% Cellvento® CHO-220 medium and cell culture grade 10% dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento® CHO-220 medium with a 1:9 volume ratio under the clean bench or laminar flow hood. As DMSO dilution will release heat during preparation, the freezing medium should be prepared in advance and stored at 2–8 °C prior to use.
- Select cells in mid-logarithmic phase and with normal shape, cell density should be > 1.5 x 10^6 cells/mL and viability >95%.
- Centrifuge at 1,200–1,500 rpm for 5 minutes (200–300 g).
- Discard the supernatant and re-suspend cells in cold freezing medium at 1 x 10^7–2 x 10^7 viable cells/mL, and transfer the cell suspension into sterile cryovials, 1 mL per vial.
- Freezing procedure with a freezing container filled with isopropanol. Place the cryovials in the cryobox and freeze the cells with a sequential decrease in temperature:
  - 30 minutes at 4 °C
  - 2–4 hours at –20 °C
  - overnight at –80 °C
  - transfer and store the vials in the liquid nitrogen tank for long-term storage

Note: The freezing procedure can be standardized using an automatic cooling instrument. In this case, the cooling speed is controlled and the cell suspension is frozen from 4 °C down to (usually) –150 °C in 1 hour.

Cell thawing and recovery procedure:

- Prepare a water bath at 37 °C for cell thawing.
- In a 50 mL centrifuge tube: prepare 10 mL culture medium under the clean bench or the laminar flow hood.
- Transfer the cryovial of CHO cells from liquid nitrogen to the 37 °C water bath.
- Take out the vial when ice particles detach from the side of the vial (DMSO may have a toxic effect at higher temperature).
- Transfer the CHO cell suspension from the cryovial to the centrifuge tube, centrifuge at 1,200–1,500 rpm for 5 minutes.
- Discard the supernatant, re-suspend the cells in fresh culture medium (Cellvento® CHO-220 medium) in order to achieve a seeding density of 3 x 10^5–5 x 10^5 cells/mL, and transfer to a 50 mL spin tube with vented cap for cultivation. Culture the cells in a 37 °C CO_2 incubator with 5% CO_2, 80% humidity and a rotation speed of 320 rpm until densities reach ≥1 x 10^6 cells/mL. Thereafter, sub-culture following standard protocols.

4. Preparation of liquid medium from powder

Reconstitution method to generate 10 L Cellvento® CHO-220 medium

1. Slowly add 203.2 grams of medium to 8 L of Milli-Q® or similar cell culture grade water in an appropriately sized container. Rinse medium container as necessary to remove remaining powder.
2. Allow to dissolve with vigorous mixing for 30 minutes (solution will still be slightly turbid).
3. Add 2 g/L sodium bicarbonate and stir until dissolved (~10 minutes).
4. Add cell culture grade water to reach a final volume of 10 L. Confirm a final pH of 7.1 ±0.3.
5. Measure the osmolality of the solution. Final osmolality should be at 310 ±40 mOsmol/kg.
6. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap or capsule filter).
7. Store at 2–8 °C protected from light. Reconstituted Cellvento® CHO-220 liquid medium is stable for at least 90 days. When supplements are added, the liquid medium is stable for max. 4 weeks.

Note: This medium does NOT contain L-glutamine. Aseptically supplement as required prior to use.

Reconstitution method to generate 3 L Cellvento® Feed-220

1. Slowly add 338.8 grams of feed to 2.5 L of Milli-Q® or similar cell culture grade water at room temperature in an appropriately sized container. Rinse feed container as necessary to remove remaining powder.
2. Vigorously mix for 45–60 minutes until fully dissolved.
3. Add cell culture grade water to reach a final volume of 3 L. Confirm final pH of 5.6 ±0.3.
4. Measure the osmolality of the solution. Final osmolality should be 1,100 ±50 mOsmol/kg. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap or capsule filter).
5. Store at 2–8 °C protected from light. Reconstituted liquid Cellvento® Feed-220 is stable for 30 days. When a bottle is opened, liquid feed is stable for max. 3 weeks.

Preparation of cysteine/tyrosine stock solution—100 mL

Component	CAS#	MW	g/L	mM
L-Cysteine	52-90-4	121.16	18.17	150
L-Tyrosine disodium salt dihydrate	122666-87-9	261.19	75.35	288.5

1. Measure 38 mL of Milli-Q® or similar cell culture grade water into an appropriate container.
2. Add 12 mL of 2 M sodium hydroxide.
3. Slowly add 1.817 g of L-cysteine and 7.535 g of L-tyrosine.
4. Adjust the pH to 11.3 ±0.1 using 5 M sodium hydroxide or 1 M hydrochloric acid and mix for 10–30 minutes to dissolve all components.
5. Add cell culture grade water to reach a final volume of 100 mL. Confirm final pH of 11.0 ±0.3.
6. Measure the osmolality of the solution. Final osmolality should be 1,220 ±40 mOsmol/kg.
7. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap filter).
8. Store at 2–8 °C protected from light. Reconstituted stock solution is stable for 1 month.

The stock solution yields concentrations of cysteine and tyrosine of 150 mM and 288.5 mM respectively, which are subsequently diluted during feeding.

To find out more about Cellvento® CHO media platform products, visit **www.MerckMillipore.com/cellvento**

5. Recommended feeding strategy

Cellvento® CHO-220 medium and companion feeds have been developed to complement each other and enhance the performance of CHO-K1 cells in protein production. As with most upstream bioprocesses, optimization of feed volumes and timing of feed administration should be empirically determined on a process- and cell-line-specific basis to maximize performance. The table on the right provides recommended ranges for evaluation of both feed volumes and frequency of feeding to optimize each parameter within the context of an overall feeding scheme.

Parameter	Recommended range for evaluation
Cellvento® Feed-220	3–6 % (v/v)
Glucose	4–6 g/L (monitor daily and maintain at 4 g/L)
Cys/Tyr feed	0.3–0.6 % (v/v) of recommended stock solution
Frequency	48–72 hour feed intervals

Recommended process guidance for initial fed-batch medium and feed evaluation in spin tubes:

Parameter	Parameter
Culture type	Spin tubes with vented cap
Initial working volume	30 mL
Inoculation density	2–3 x 10^5 cells/mL
Agitation rate	320 rpm
Production medium	Cellvento® CHO-220 chemically defined cell culture medium
Main feed	Cellvento® Feed-220 chemically defined cell culture feed
Feed supplement	Cys/Tyr stock solution
Temperature	37.0 ±0.5 °C
Incubator CO_2	5%
Media pH	7.1 ±0.3
Harvest criterion	End culture when viability <50–70%
Sampling points	Study days 0, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13, 14
Cellvento® Feed-220 volume	See table above
Cys/Tyr stock solution	See table above
Feeding schedule	See table below
Glucose feed addition	Daily addition, maintaining concentration above 1 g/L and levels post-feeding at 4–6 g/L

Although we recommend sampling the culture on day 0 to confirm the seeding density, the first proposed post-inoculation sampling time point is study day 3, followed by daily sampling. Minimal sampling volume (i. e., <800 µL) is recommended.

Measurement parameters on sampling days:

- Viable cell density
- Viability
- Glucose, glutamine (as appropriate)
- Recombinant protein product

Suggested initial feeding evaluation

Initiate the feeding only when viable cell density is ≥2 x 10^6 cells/mL and no earlier than day 3 (to avoid over-feeding).

Maintain supplementation with feed supplements and glucose until culture viability is less than 80%. Terminate and harvest cultures when viability drops below 50–70%.

Culture day	Addition order	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cellvento® Feed-220 (% v/v)	1				3		6		6			6				
Glucose	2	Monitor daily and maintain at 4–6 g/L														
Cys/Tyr stock solution (% v/v)*	3			0.3		0.6		0.6			0.6					

* Add to culture slowly

Fed-batch performance in spin tubes

To confirm media performance and consistency over different lots of Cellvento® CHO-220 production medium, performance trials were run in spin tubes on a recombinant CHO-K1 cell line. Cell growth and protein production profiles were indistinguishable in the 3 production lots tested, ensuring that Cellvento® CHO-220 medium can be used confidently with minimal risk of process variability attributable to cell culture media raw materials.

Clone 1

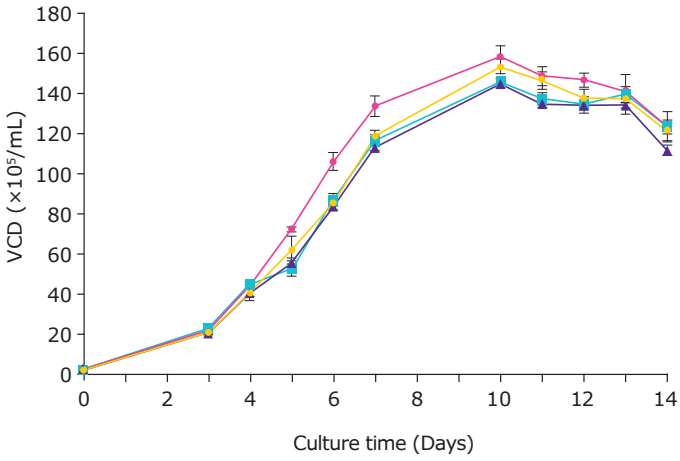

Figure 1: Growth profiles in fed-batch culture. CHO-K1 cells (clone 1) were grown in Cellvento® CHO-220 medium supplemented with complementary Cellvento® Feed products.

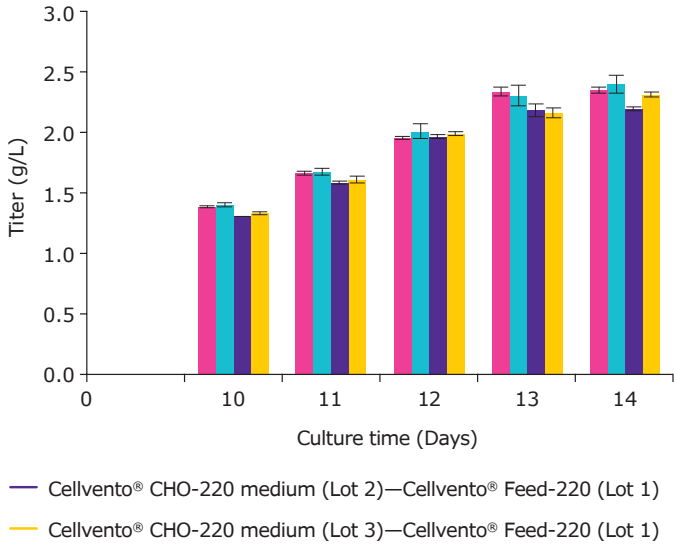

Figure 2: Consistent IgG titers were achieved using three different lots of Cellvento® CHO-220 medium against one lot of Cellvento® Feed-220 over a 14-day fed-batch culture with CHO-K1 cells (clone 1).

Clone 2

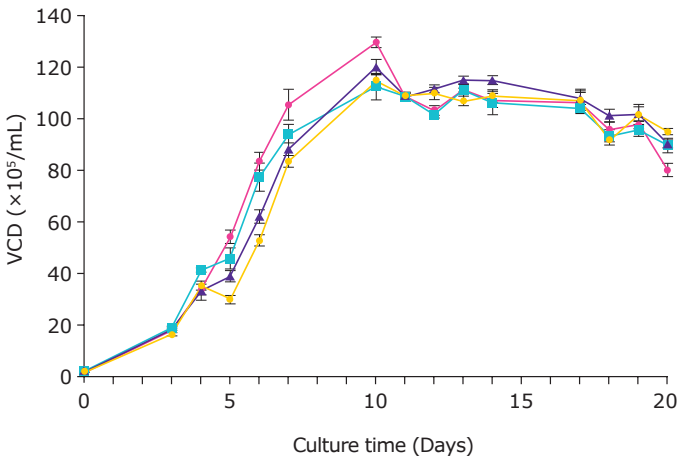

Figure 3: Growth profiles in fed-batch culture. CHO-K1 cells (clone 2) were grown in Cellvento® CHO-220 medium supplemented with complementary Cellvento® Feed products.

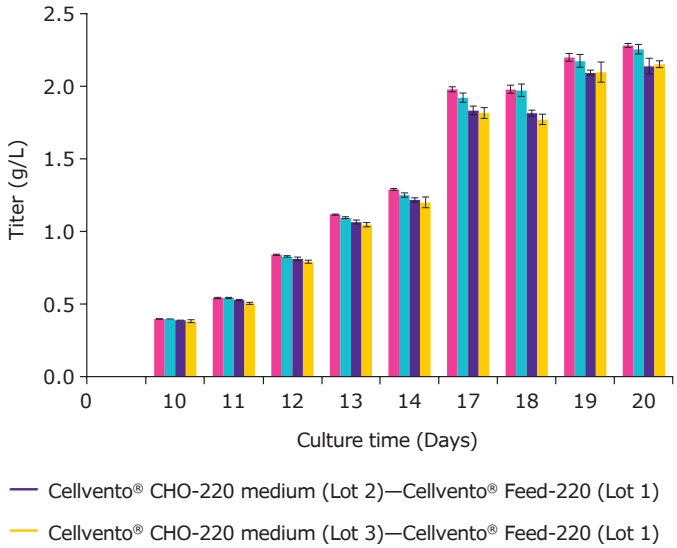

Figure 4: Consistent IgG titers were achieved using three different lots of Cellvento® CHO-220 medium against one lot of Cellvento® Feed-220 over a 14-day fed-batch culture with CHO-K1 cells (clone 2).

Troubleshooting

No.	Question/Problem	Reason/Solution
1	The liquid medium is still cloudy or hazy after the recommended mixing time during the first reconstitution step.	Depending on the agitation rate or mixing process and water temperature, there may still be some haziness following the first mixing step. This haziness will dissipate when adding sodium bicarbonate.
2	Can I add L-glutamine prior to the sterile filtration step in order to prepare a complete medium?	Yes. You may add powder or liquid L-glutamine during the first mixing step, and prior to the initial pH adjustment. Complete media supplemented with L-glutamine should be used within 60 days to minimize impact on stability and ammonia accumulation.
3	Do I need to supplement the medium with poloxamer prior to use?	No. Cellvento® CHO-220 medium contains 2.0 g/L poloxamer, which is sufficient to protect suspension cultures from sheer stress. Adding additional poloxamer may adversely impact cell growth and cause problems in downstream processing and purification steps.
4	Can I use nylon-based filters for the media filtration?	No. We recommend the use of 0.22 µm Millipore Express® PLUS or Durapore® membrane filters. Nylon or cellulose-acetate-based filter membranes may non-specifically bind critical media components and adversely impact performance.
5	Can I use Cellvento® CHO-220 medium with cells in 8–10% CO_2 incubators?	Cellvento® CHO media have been optimized for use with 5% CO_2 incubation. You may need to increase the sodium bicarbonate concentration to offset and minimize the impact of the higher carbonic acid levels and decreased media pH on the cultures.
6	The osmolality of the complete Cellvento® CHO-220 medium prior to filtration is >355 mOsmol/kg.	We recommend preparing fresh media, as we typically observe (with multiple batches and media lots) final media osmolalities of 310–315 mOsmol/kg in both our R&D and QC labs. An out-of-specification media osmolality is typically the result of a misformulation or multiple acid/base titrations during the pH adjustment steps.
7	Why do I need to store the complete liquid media protected from light?	Cellvento® CHO media and feed supplements contain light-sensitive components like vitamins which are rapidly oxidized upon fluorescent light exposure, resulting in decreased stability and cellular performance.
8	There is a precipitate in the medium or feed supplement following extended storage at 2–8 °C.	Prepare fresh medium or feed supplements. Cellvento® CHO media and feed supplements contain components at high concentrations that are required to support high-density fed-batch cell culture applications. Components may come out of solution with time and/or following multiple uses and warming/cooling steps. Use Cellvento® CHO-220 medium and Cellvento® Feed-220 supplement within 90 days and 30 days of preparation, respectively.
9	We observe rapid cell growth but low protein expression or antibody titers in fed-batch cultures using Cellvento® CHO-220 medium.	Fed-batch cultures should reach 3- to 5-fold higher protein levels or antibody titers vs. batch culture concentrations. This is either the result of a low-expressing cell line or a nutrient rate limitation during the production phase of the fed-batch culture. Measure and maintain glucose concentrations at 4–6 g/L and optimize the feed addition timing and volume additions for Cellvento® Feed-220 and the cysteine/tyrosine feed supplements.

The typical technical data above serve to generally characterize the cell culture media in industry-relevant expression systems. The product information is available separately from the website: **www.MerckMillipore.com**

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