Process Guidance

Cellvento™ CHO-200
Chemically defined cell culture medium for fed-batch application

The purpose of a fed-batch process in cell culture is two-fold: firstly, a production medium is used to support initial cell growth and production, and secondly, a feed medium is added to replenish depleted nutrients required for cellular function and to maintain the production phase of the culture. Because the performance of production media and their companion feed(s) is typically inter-dependent, optimizing a feeding strategy is crucial to achieving a culture that both grows and produces effectively. While recommended ranges of feed volumes and frequencies are typically provided with media and feed products, optimal feed conditions typically need to be empirically determined for a given cell line and its associated bioprocess. This document provides the basis for initiating feed optimization activities, but fine-tuning an effective feeding strategy should be considered.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

The fed-batch media system

Cellvento™ CHO-200 medium and its companion Cellvento™ Feed-200 are chemically-defined, non-animal origin products designed for use in CHO-S cell-based mammalian cell culture. The medium and its feeds are effective at achieving high-density cell growth and competitive productivity in the CHO-S GS expression platform, but may also be appropriate for use with other CHO-S cell lines. As in all fed-batch processes, however, optimization of feeding volumes and frequency is required.

Production medium and main feed

1.01885.0010	Cellvento™ CHO-200 medium
1.01883.0003	Cellvento™ Feed-200

Additional feed supplements

1.02735.0100	L-Cysteine HCl monohydrate EMPROVE® exp
1.02413.0100	L-Tyrosine disodium salt dihydrate
1.02415.0400	Glucose for cell culture media

Additives

1.37013.1000	Sodium hydrogen carbonate
1.00286.1000	L-Glutamine EMPROVE® exp
F 0493	HT (50x) supplement

All components are available individually.

Applications

- Cellvento™ CHO-200 medium and its feeds have been designed for use in the CHO-S GS expression system in cell suspension culture, but may be suitable for other CHO-S cell lines.
- Cellvento™ CHO-200 medium should be used for cell adaptation and cell bank generation
- Cellvento™ CHO-200 medium is suitable for use in seed train expansion
- Cellvento™ products allow for flexibility in feed and feed supplement optimization of fed-batch processes.

Using Cellvento™ CHO-200 medium in fed-batch mode

- Supplement Cellvento™ CHO-200 medium with 100 µM hypoxanthine and 16 µM thymidine for all non-dihydrofolate reductase (DHFR) amplified cell lines. This can be accomplished by adding 20 mL/L HT (50x) supplement.
- Add 6 mM L-Glutamine to Cellvento™ CHO-200 medium prior to use with non-GS CHO cells lines. Cellvento™ Feed-200 does not require any additional supplementation with L-Glutamine for use in fed-batch culture.
- Glucose should be added separately during feeding to maintain appropriate levels throughout the fed-batch culture.
- Cell selection agents should be added as required.
- Optimal volumes and timing of Cellvento™ Feed-200 and Cys/Tyr feed administration should be determined experimentally.

Options for Cellvento™ CHO-200 media system evaluation

1. Direct media adaptation of CHO-S cells

Some cells may adapt directly into Cellvento™ CHO-200 medium. Cells should be seeded at $2.5x10^5$ –$3.5x10^5$ cells/mL, then sub-cultured when densities reach $1x10^6$–$3x10^6$ cells/mL and ≥ 90% viability. Adaptation is complete when cells maintain normal doubling time and VCD ≥ 90% over at least 2 passages.

2. Sequential media adaptation of CHO-S cells

Typically cells should be passaged every 3 to 4 days in order to maintain logarithmic growth. At least two passages at each adaptation step are recommended to ensure that cells appropriately adjust to their new media environments.

Ratio of current media to Cellvento™ CHO-200 medium (in %)	Seeding density ($x10^5$ 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; VCD ≥ 90% over at least 2 passages
50:50	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; VCD ≥ 90% over at least 2 passages
25:75	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; VCD ≥ 90% 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; VCD ≥ 90% over at least 2 passages

3. Cryopreservation

Viable cell banks may be created by freezing cells in 90% Cellvento™ CHO-200 and 10% dimethyl sulfoxide (DMSO).

Freezing:

- Cultures should be in logarithmic phase and at least 90% viable for cryopreservation.
- Prepare the freezing medium by combining Cellvento™ CHO-200 at 90% with dimethyl sulfoxide (DMSO) at 10%. Store at 2–8 °C until use.
- Precipitate cells by centrifugation at 1,000 rpm for 5 minutes (100–200 xg (rcf)).
- Decant supernatant and resuspend cells in cold freezing medium at $5 x 10^6$–$1 x 10^7$ viable cells/mL.
- Transfer aliquots of the cell suspension into sterile cryovials.
- Freeze cells at –80 °C for 24 hours and then transfer to liquid nitrogen for long-term storage.

Thawing:

- Thaw a vial of frozen cells rapidly in a 37 °C water bath.
- Transfer cells to a centrifuge tube with 10 mL of Cellvento™ CHO-200 medium at room temperature.
- Precipitate cells by centrifugation at 1,000 rpm for 5 minutes.
- Decant supernatant and resuspend cells in an adequate volume of Cellvento™ CHO-200 media. Seed a shaker flask at $3 x 10^5$–$5 x 10^5$ cells/mL.
- Incubate cells at 37 °C and 5% CO_2 until densities reach $1 x 10^6$ cells/mL. Thereafter, sub-culture following standard protocols.

4. Transformation from powder to liquid medium

Reconstitution method to generate 10 L Cellvento™ CHO-200 medium

1. Slowly add 232 grams of powder to 8.0 L of Milli-Q® or similar cell culture grade water in an appropriately sized container.
2. Allow to dissolve by gently stirring for 45–60 minutes (solution will still be slightly turbid). Then adjust pH to 5.5 +/– 0.2 using 5 M sodium hydroxide.
3. Add 2 g/L sodium bicarbonate and stir until dissolved (~10 minutes).
4. Adjust the pH to 7.0 +/– 0.2 using 5 M sodium hydroxide or 1 M hydrochloric acid if needed.
5. Add cell culture grade water to reach a final volume of 10 L and confirm a final pH of 7.0 +/– 0.2.
6. Measure the osmolality of the solution. Final osmolality should be 315 +/– 10 mOsmol/kg.
7. Sterilize by membrane filtration using a 0.2 µm filter.
8. Store at 2–8 °C protected from light.

Reconstitution method to generate 3L Cellvento™ Feed-200

1. Add 347 grams of powder to 2.4 L of Milli-Q® or similar cell culture grade water in an appropriately sized container.
2. Stir until fully dissolved (45–60 minutes) then adjust the pH to 5.6 ± 0.1 using 5 M sodium hydroxide or 1 M hydrochloric acid if needed.
3. Add cell culture grade water to reach a final volume of 3L and confirm a final pH of 5.6 ± 0.1.
4. Measure the osmolality of the solution. Final osmolality should be 1,100 ± 50 mOsmol/kg. Sterilize by membrane filtration using a 0.2 µm filter
5. Store at 2–8 °C protected from light.

Preparation of Cys/Tyr stock solution – 150 mL

Component	CAS#	FW	g / Liter	mM
L-Cysteine hydrochloride monoydrate	7048-04-6	175.63	52.67	299.90
L-Tyrosine disodium salt dihydrate	122666-87-9	261.19	149.64	573.00

1. Measure 0.1 L of Milli-Q® water or similar cell culture grade water into an appropriate container and adjust the pH ≥ 13 using 5 M sodium hydroxide.
2. Slowly add 7.9 g of L-Cysteine and 22.45 g of L-Tyrosine to the beaker.
3. Adjust the pH to 11.3 ± 0.1 using 5 M sodium hydroxide or 1 M hydrochloric acid and mix for 10 minutes to dissolve all components.
4. Add cell culture grade water to reach a final volume of 0.15 L, then confirm the pH is 11.3 ± 0.1.
5. Measure the osmolality of the solution. Final osmolality should be 3100 ± 100 mOsmol/kg.
6. Sterilize by membrane filtration using a 0.2 µM and store at 2–8 °C protected from light.
7. The generation of this stock solution yields concentrations of cysteine and tyrosine of 300 and 573 mM respectively, which are subsequently diluted during feeding.

Additional information about the products is available on www.cellvento.com

5. Recommended feeding strategy

Cellvento™ CHO-200 medium and companion feeds have been developed to complement each other and enhance the performance of CHO-S cells in protein production. As with most upstream bioprocesses, application of feed volumes and timing of administration should be empirically determined on a process- and cell-line-specific basis to maximize performance.

The table below 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-200	2–6% (v/v)
Glucose	2–4 g/L (monitor daily and supplement as needed to maintain)
Cys/Tyr Feed	0.15–0.3% (v/v) of recommended stock solution
Frequency	48–72 hour feed intervals

Recommended process guidance for initial fed-batch medium and feed evaluation in shaker flasks:

Experimental condition	Operating Parameter
Culture type	125 mL shaker flask
Initial working volume	45 mL
Inoculation density	$3x10^5$ cells/mL
Agitation rate	150 rpm (25mm orbital)
Production medium	Cellvento™ CHO-200 Chemically defined cell culture medium
Feed medium	Cellvento™ Feed-200 Chemically defined cell culture feed
Feed supplement	Cys/Tyr stock solution
Temperature	37.0 ± 0.5 °C
Incubator pCO_2	5%
Media pH	7.0
Harvest criterion	End culture when viability < 75%
Sampling points	Study days 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
Cellvento™ Feed-200 volume	See table above
Cys/Tyr stock solution	See table above
Feeding schedule	See table above
Glucose feed addition	Daily addition, maintaining levels ≥ 4 g/L

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.

Measuring parameters at sampling days:

- viable cell density
- viability, glucose
- glutamine (as appropriate)
- recombinant protein product

Suggested initial feeding evaluation options

Feeding option 1

Culture day	Addition order	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cellvento™ Feed-200 (% v/v)	1				3		6		6		6					
Glucose	2	monitor daily and maintain at 4 g/L daily														
Cys/Tyr stock solution (% v/v)	3*				0.15		0.3		0.3		0.3					

Glucose is a bolus addition base requiring daily monitoring after day 3. One option is to supplement with a sterile 40% glucose solution.

*Add to culture slowly

Feeding option 2

Culture day	Addition order	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cellvento™ Feed-200 (% v/v)	1				3		6		6		6		3*			
Glucose	2	monitor daily and maintain at 4 g/L daily														
Cys/Tyr stock solution (% v/v)	3*				0.15		0.15		0.15		0.15		0.07**			

*Add to culture slowly

**Feed only if culture viability ≥ 90%

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.emdmillipore.com**

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