SAFC®
Pharma & Biopharma Raw Material Solutions

Product Information

Cellvento® CHO-200

Chemically defined cell culture medium

Product description

Cellvento® CHO-200 chemically defined cell culture medium has been specially developed for the growth of Chinese Hamster Ovary (CHO) cells and the expression of monoclonal antibodies and recombinant proteins in suspension culture. The formulation is of non-animal origin, chemically defined and contains no hydrolysates or components of unknown composition.

Cellvento® CHO-200 medium has been formulated without L-glutamine, hypoxanthine, and thymidine to keep flexibility in applications. It is available in dry powder form or as ready-to-use medium to fit to different experimental set-ups.

Application

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.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Media preparation

Supplement Cellvento® CHO-200 medium with 100 µM hypoxanthine and 16 µM thymidine when using parental dihydrofolate reductase deficient cell lines (DHFR-) and for all non-dihydrofolate reductase amplified cell lines. This can be accomplished by adding 20 mL/L HT (50 x) supplement.

Aseptically add 4 – 8 mM L-glutamine to Cellvento® CHO-200 medium prior to use with non-GS CHO cell lines.

Supplementation with a surfactant (e.g., poloxamer) is not required to use this product.

Cell selection agents should be added as required prior to use. In general, we recommend removing the selective pressure agent from the final batch production step and culture.

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Reconstitution method to prepare 10 L Cellvento® CHO-200 medium

1. Slowly add 232 g of powder to 8.0 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 gentle stirring for 45-60 minutes (solution will still be slightly turbid). 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. Confirm a final pH of 7.0 ± 0.2.
6. Measure the osmolality of the solution. Final osmolality should be at 315 ± 15 mOsmol/kg.
7. Immediately filter using a sterilizing-grade filter (≤0.22µm). For filter recommendations, see Page 4.
8. Store at 2–8 °C protected from light. Reconstituted Cellvento® CHO-200 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, hypoxanthine, or thymidine. Aseptically supplement as required prior to use. After filtration of powder medium, use appropriate aseptic techniques when handling or supplementing this medium.

Storage

Dry powder should be stored at 2–8 °C protected from light.

Do not use after expiration date.

Direct media adaptation

Some cells may be adapted directly into Cellvento® CHO-200 medium. Cells should be seeded at 2×10^5 – 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.

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 two 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-200 medium (in %)	Seeding density (× 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

Cryopreservation

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

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento® CHO-200 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 $>1.5\times10^6$ cells/mL and viability > 95 %.
- Centrifuge at 1,200–1,500 rpm for 5 minutes (200–300 g).
- Discard the supernatant and resuspend cells in cold freezing medium at 5×10^6 – 1×10^7 viable cells/mL, and transfer the cell suspension into sterile cryovials, 1 mL per vial.
- Freezing procedure with a freezing container containing 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, resuspend the cells in fresh culture medium (Cellvento® CHO-200 medium) in order to achieve a seeding density of 2×10^5 – 5×10^5 cells/mL, and transfer to a vented cap 125 mL Erlenmeyer flask for cultivation. Culture the cells in a 37 °C CO_2 incubator with 5 % CO_2, 80 % humidity and a rotation speed of 110 rpm until densities reach $\geq1\times10^6$ cells/mL. Thereafter, sub-culture following standard protocols.

Ordering Information

Cat. No.	Product Name	Pkg. size
Cellvento® CHO-200 medium – Dry powder		
1.01885.0010	Cellvento® CHO-200 Chemically defined cell culture medium	0.232 kg (10 L)
1.01885.0100	Cellvento® CHO-200 Chemically defined cell culture medium	2.325 kg (100 L)
Companion Cellvento® Feed-200		
1.01883.0003	Cellvento® Feed-200 Chemically defined cell culture feed	0.347 kg
1.01883.0010	Cellvento® Feed-200 Chemically defined cell culture feed	1.158 kg
1.01883.0050	Cellvento® Feed-200 Chemically defined cell culture feed	5.791 kg
Cell culture additives		
1.00286.1000	L-Glutamine suitable for use as excipient EMPROVE® exp DAB, USP	1 kg
1.37013.2500	Sodium hydrogen carbonate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JP	2.5 kg
1.02413.0100	L-Tyrosine disodium salt dihydrate for cell culture media	0.1 kg
1.02735.0100	L-Cysteine hydrochloride monohydrate suitable for use as excipient EMPROVE® exp Ph Eur, USP	0.1 kg

Ordering Information for sterilizing-grade filters

	Bacteria Removal	Mycoplasma & Bacteria Removal
Volume	Millipore Express® SHC	Millipore Express® SHR with Prefilter
10 L	KHGES015FF3	KHVES015FF3
100 L	KHGES03TT3	KHVES03TT3

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