SAFC®
Pharma & Biopharma Raw Material Solutions

Product Information

Cellvento® CHO-110

Chemically defined cell culture medium

Product description

Cellvento® CHO-110 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-110 medium has been formulated without L-glutamine, hypoxanthine, and tymidine 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-110 medium:

- has been designed to support optimized cell growth and performance with DHFR-negative CHO suspension cell types, primarily recombinant CHO-DG44 cell lines, but may be suitable for use with other CHO cell lines.
- should be used for cell adaptation and cell bank generation.
- is suitable for production in batch and perfusion culture applications.
- is suitable for seed train expansion up to the N-2 step for fed-batch processes, switching to Cellvento® CHO-210 medium at the final N-1 expansion step, which is then used in conjunction with Cellvento® Feed-210 companion feed in the final production culture.

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

Media preparation

Supplement Cellvento® CHO-110 medium with 100 µM hypoxanthine and 16 µM thymidine for 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-110 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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Transformation from powder to liquid medium

Reconstitution method to prepare 10 L Cellvento® CHO-110 medium

1. Slowly add 275 grams 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 vigorous mixing for 30 minutes (solution will still be slightly turbid).
   Adjust pH to 6.2 ± 0.1 using 5 M sodium hydroxide (typically requires ~1.5–2 mL/L to reach target pH).
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 ± 30 mOsmol/kg.
7. Immediately filter using a sterilizing-grade filter (≤ 0.22µm). For filter recommendation, see Page 4.
8. Store at 2–8 °C protected from light. Reconstituted Cellvento® CHO-110 liquid medium is stable for at least 60 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. 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.

Liquid medium should be stored at 2-8 °C protected from light.

Do not use after expiration date.

Direct media adaptation

Cell lines may be adapted directly into Cellvento® CHO-110 medium. Cells should be seeded at $3x10^5$–$5x10^5$ cells/mL, then sub-cultured when densities reach $1x10^6$–$3x10^6$ cells/mL and ≥ 80 % viability. Adaptation is complete when cells attain a stable doubling time (20–30 hours) and VCD ≥ 90 % over at least 2–3 passages.

Cells that are initially adapted to and cultured in Cellvento® CHO-110 growth medium can be sub-cultured directly into Cellvento® CHO-210 medium.

Cells banked in Cellvento® CHO-110 medium should be thawed and maintained in Cellvento® CHO-110 growth medium for at least 2 passages prior to sub-culturing in Cellvento® CHO-210 medium.

Remark: Cellvento® CHO-210 (Art. No. 102485) serves as production medium in fed-batch processes. Further information is available in a separate Product Information.

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-110 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-110 medium and cell culture grade 10 % dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento® CHO-110 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 $>1.5x10^6$ 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 $1x10^7$–$2x10^7$ 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-110 medium) in order to achieve a seeding density of $3x10^5$–$5x10^5$ 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 $\geq 1x10^6$ cells/mL. Thereafter, sub-culture following standard protocols.

Ordering Information

Cat. No.	Product Name	Pkg. size
Cellvento® CHO-110 medium – Dry powder		
1.02482.0010	Cellvento® CHO-110 Chemically defined cell culture medium	275.0 g (10 L)
1.02482.0100	Cellvento® CHO-110 Chemically defined cell culture medium	2.750 kg (100 L)
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

Ordering Information for sterilizing-grade filters

	Bacteria Removal	Mycoplasma & Bacteria Removal	Virus, Mycoplasma & Bacteria
Volume	Millipore® Express SHC	Millipore® Express SHC with Prefilter	Viresolve® Barrier
1 L	KHGES015FF3	KHVES015FF3	VBKG005TC1
10 L	KHGES015FF3	KHVES015FF3	VBKG015TC1
100 L	KHGES003FF3	KHVES006FF3	VBKG050TC1

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