Product Information

Cellvento™ CHO-210 Chemically defined cell culture medium

Product description

Cellvento™ CHO-210 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 for use in fed-batch manufacturing processes. The formulation is of non-animal origin, chemically defined and contains no hydrolysates or components of unknown composition.

Cellvento™ CHO-210 medium has been formulated without L-glutamine, hypoxanthine, thymidine, or thymidine, to keep flexibility in applications. It is available in dry powder from or as ready-to-use medium to fit to different experimental set-ups.

Application

Cellvento™ CHO-210 medium has been designed to support optimal cell growth and performance of DHFR-negative CHO suspension cell types, primarily recombinant CHO-DG44 cell lines, but may be suitable for use with other CHO cell lines. It should be used as a production medium starting with the final N-1 expansion step in fed-batch applications, and then together with its companion feed product Cellvento™ Feed-210 during the final production culture.

We recommend using Cellvento™ CHO-110 growth medium for seed train expansion up to the N-1 step, as it is a richer formulation than Cellvento™ CHO-210 medium and does not require additional supplementation.

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

Media preparation

Supplement Cellvento™ CHO-210 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 ×) supplement.

Aseptically add 4–8 mM L-glutamine to Cellvento™ CHO-210 medium prior to use with non-GS CHO cells 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 during the final fed-batch production step or culture.

Transformation from powder to liquid medium

Reconstitution method to prepare 10 L Cellvento™ CHO-210 medium

1. Slowly add 231 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 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 +/– 40 mOsmol/kg.
7. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap or capsule filter).
8. Store at 2–8 °C protected from light.
   Reconstituted Cellvento™ CHO-210 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. Liquid medium should be stored at 2-8 °C protected from light. Do not use after expiration date.

Shelf life

Dry powder medium: 12 months
Liquid medium: 12 months

Direct media adaptation

Cell lines may be adapted directly into Cellvento™ CHO-210 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.

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-210 medium (in %)	Seeding density ($\times 10^5$ 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-210 medium and cell culture grade 10 % dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento™ CHO-210 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 1×10^7–2×10^7 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.

Ordering information for Cellvento™ CHO-210 medium – Dry powder

Catalog number	Product name	Pkg. size	Equivalent
1.02485.0010	Cellvento™ CHO-210 Chemically defined cell culture medium	231.3 g	10 liters
1.02485.0100	Cellvento™ CHO-210 Chemically defined cell culture medium	2.313 kg	100 liters

Ordering information for companion Cellvento™ Feed-210

Catalog number	Product name	Pkg. size	Equivalent
1.02488.0005	Cellvento™ Feed-210 Chemically defined cell culture feed	408 g	5 liters
1.02488.0050	Cellvento™ Feed-210 Chemically defined cell culture feed	4.080 kg	50 liters

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

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-210 medium) in order to achieve a seeding density of 3×10^5–5×10^5 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 for Cellvento™ CHO-210 medium – Liquid

Catalog number	Product name	Pkg. size
1.02553.0500	Cellvento™ CHO-210 Liquid Chemically defined cell culture medium	500 mL
1.02553.1000	Cellvento™ CHO-210 Liquid Chemically defined cell culture medium	1,000 mL

Ordering information for cell culture additives

Catalog number	Product name	Pkg. size
1.00286.1000	L-Glutamine suitable for use as excipient EMPROVE® exp DAB, USP	1 kg
1.37013.2500	Sodium hydrogen carbonate suitable for the biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JP	2.5 kg
Available on request	HT Supplement (50 ×)	100 mL
1.02735.0100	L-Cysteine hydrochloride monohydrate suitable for use as excipient EMPROVE® exp Ph Eur, USP	100 g
1.02413.0100	L-Tyrosine disodium salt dihydrate for cell culture media	100 g
1.02415.0400	D(+)-Glucose anhydrous for cell culture media	400 g

Ordering information for aseptic filters

Catalog number	Product name	Pkg. size
GPWP02500	Millipore Express® PLUS Membrane, 0.22 µm, 25 mm	100
GVWP02500	Durapore® Membrane, 0.22 µm, 25 mm	100

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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