

Product Information

Cellvento™ CHO-100

Chemically defined cell culture medium

Product description

Cellvento™ CHO-100 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-100 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-100 medium:

- has been designed to support optimized cell growth and performance with CHO-S suspension cell types, but may be suitable for use with other commonly used CHO cell lines.
- should be used for cell adaptation, cell bank generation, and transfection.
- 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-200 medium at the final N-1 expansion step, which is then used in conjunction with Cellvento™ Feed-200 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-100 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 (50x) supplement. Aseptically add 4–8 mM L-glutamine to Cellvento™ CHO-100 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.

Transformation from powder to liquid medium

Reconstitution method to

prepare 10 L Cellvento™ CHO-100 medium

1. Slowly add 269 g of powder to 9.5 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 mixing for 60 minutes. Adjust pH to 6.9 +/- 0.1 using 5 M sodium hydroxide (typically requires ~1.8 mL/L to reach target pH).
3. Add 1.2 g/L sodium bicarbonate and stir until dissolved (~10 minutes).
4. Adjust the pH to 7.1 +/- 0.1 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 7.1 +/- 0.1.
6. Measure the osmolality of the solution. Final osmolality should be at 315 +/- 15 mOsmol/kg.

Direct media adaptation

Cell lines may be adapted directly into Cellvento™ CHO-100 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 $\geq 80\%$ viability. Adaptation is complete when cells attain a stable doubling time (20 – 30 hours) and viability is $\geq 90\%$ over at least 2 – 3 passages.

Cells that are initially adapted to and cultured in Cellvento™ CHO-100 growth medium can be sub-cultured directly into Cellvento™ CHO-200 medium. Cells banked in Cellvento™ CHO-100 medium should be thawed and maintained in Cellvento™ CHO-100 growth medium for at least 2 passages prior to sub-culturing in Cellvento™ CHO-200 medium.

7. Sterilize by membrane filtration using a 0.22 μ m Millipore Express® PLUS or Durapore® membrane, bottle cap, or capsule filter.
8. Store at 2 – 8 °C protected from light.

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 medium 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: 24 months

Liquid medium: 12 months

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-100 medium (in %)	Seeding density ($\times 10^5$ cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
75:25	2.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; viability > 80% over at least 2 passages
50:50	2.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; viability > 80% over at least 2 passages
25:75	2.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; viability > 80% over at least 2 passages
0:100	2.0	Cell density, viability in mid-log growth phase	Adaptation complete when cells maintain normal doubling time; viability $\geq 90\%$ over at least 2 passages

Cryopreservation

Viable cell banks may be created by freezing cells in 90% Cellvento™ CHO-100 medium and cell culture grade 10% dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento™ CHO-100 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 $> 1.5 \times 10^6$ cells/mL and viability $> 95\%$.
- Centrifuge at 1,200–1,500 rpm for 5 minutes (200–300g).
- 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-100 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 CO₂ incubator with 5% CO₂, 80% humidity and a rotation speed of 110 rpm until densities reach $\geq 1 \times 10^6$ cells/mL. Thereafter, sub-culture following standard protocols.

Ordering information for Cellvento™ CHO-100 medium – Dry powder

Catalog number	Product name	Pkg. size	Equivalent
1.00899.0010	Cellvento™ CHO-100 Chemically defined cell culture medium	0.269 kg	10 liters
1.00899.0100	Cellvento™ CHO-100 Chemically defined cell culture medium	2.696 kg	100 liters

Ordering information for Cellvento™ CHO-100 medium – Liquid

Catalog number	Product name	Pkg. size
1.02454.0500	Cellvento™ CHO-100 Liquid Chemically defined cell culture medium	500 mL
1.02454.1000	Cellvento™ CHO-100 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 biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JP	2.5 kg
Available on request	HT Supplement (50 ×)	100 mL

Ordering information for aseptic filters

Catalog number	Product name	Qty/Pk
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

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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