Product Information

Cellvento™ CHO-220
Chemically defined cell culture medium

Product description

Cellvento™ CHO-220 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-220 medium has been formulated without L-glutamine. It contains hypoxanthine and thymidine, and is available in dry powder form or as ready-to-use medium to fit to different experimental set-ups.

Application

Cellvento™ CHO-220 medium and its companion feeds have been designed for use with recombinant CHO-K1 suspension cells, but may also be suitable for other CHO cell lines.

- Cellvento™ CHO-220 medium should be used as an amplification and production medium in fed-batch applications (together with its companion feed product Cellvento™ Feed-220).
- 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

Aseptically add 4 – 8 mM L-glutamine to Cellvento™ CHO-220 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.

Preparation of liquid medium from powder

Reconstitution method to generate 10 L Cellvento™ CHO-220 medium

1. Slowly add 203.2 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 vigorous mixing for 30 minutes (solution will still be slightly turbid).
3. Add 2 g/L sodium bicarbonate and stir until dissolved (~10 minutes).
4. Add cell culture grade water to reach a final volume of 10 L. Confirm a final pH of 7.1 +/- 0.3.

5. Measure the osmolality of the solution. Final osmolality should be at 310 +/– 40 mOsmol/kg.
6. Sterilize by membrane filtration using a 0.22 µm Millipore Express® PLUS or Durapore® membrane filter (bottle cap or capsule filter).
7. Store at 2 – 8 °C protected from light.
   Reconstituted Cellvento™ CHO-220 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. 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: 12 months
Liquid medium: 12 months

Direct media adaptation

Cell lines may be adapted directly into Cellvento™ CHO-220 medium. Cells should be seeded at 3×10^5 – 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.

Cells that are initially adapted to and cultured in Cellvento™ CHO-100 or Cellvento™ CHO-110 growth medis can be sub-cultured directly into Cellvento™ CHO-220 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-220 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 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-220 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-220 medium and cell culture grade 10 % dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento™ CHO-220 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 > 1.5×10^6 cells/mL and viability > 95 %.
- Centrifuge at 1,200 – 1,500 rpm for 5 minutes (200 – 300 g).
- Discard the supernatant and re-suspend cells in cold freezing medium at $1 \times 10^7 - 2 \times 10^7$ viable cells/mL, and transfer the cell suspension into sterile cryovials, 1 mL per vial.
- Freezing procedure with a freezing container filled with 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, re-suspend the cells in fresh culture medium (Cellvento™ CHO-220 medium) in order to achieve a seeding density of $3 \times 10^5 - 5 \times 10^5$ cells/mL, and transfer to a 50 mL spin tube with vented cap for cultivation. Culture the cells in a 37 °C CO_2 incubator with 5 % CO_2, 80 % humidity and a rotation speed of 320 rpm until densities reach ≥ 1×10^6 cells/mL. Thereafter, sub-culture following standard protocols.

Ordering information for Cellvento™ CHO-220 medium – Dry powder

Catalog number	Product name	Pkg. size	Equivalent
1.02557.0010	Cellvento™ CHO-220 Chemically defined cell culture medium	0.203 kg	10 liters
1.02577.0100	Cellvento™ CHO-220 Chemically defined cell culture medium	2.032 kg	100 liters

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
1.02413.0100	L-Tyrosine disodium salt dihydrate for cell culture media	100 g
1.02452.0025	L-Cysteine for cell culture media	25 g
1.02415.0400	D(+) Glucose anhydrous for cell culture media	400 g

Ordering information for Cellvento™ CHO-220 medium – Liquid

Catalog number	Product name	Pkg. size
1.02680.0500	Cellvento™ CHO-220 Liquid Chemically defined cell culture medium	500 mL
1.02680.1000	Cellvento™ CHO-220 Liquid Chemically defined cell culture medium	1,000 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

Ordering information for Cellvento™ Feed-220

Catalog number	Product name	Qty/Pk
1.02578.0003	Cellvento™ Feed-220 Chemically defined cell culture feed	0.339 kg
1.02578.0010	Cellvento™ Feed-220 Chemically defined cell culture feed	1.129 kg
1.02578.0050	Cellvento™ Feed-220 Chemically defined cell culture feed	5.647 kg

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

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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For more information and documentation please contact:

Phone: +49 6151 72-0
Email: pcs.salessupportEU@merckgroup.com

Merck Millipore
Merck KGaA
Frankfurter Str. 250
64293 Darmstadt, Germany

www.merckmillipore.com