SAFC®
Pharma & Biopharma Raw Material Solutions

Cellvento® 4CHO

Chemically defined cell culture medium

Product description

Cellvento® 4CHO 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® 4CHO medium has been formulated without L-glutamine.

Application

Cellvento® 4CHO medium should be used as amplification medium and production medium in fed-batch applications. It is recommended to combine it with the companion feed supplement Cellvento® 4Feed in fed-batch manufacturing processes.

The medium does not contain hypoxanthine and thymidine to allow a broad utilization, also in dhfr- transfected cells. The feed does not contain glucose to allow a fine tuning of the glucose concentration during fed-batch processes, thus allowing to minimize lactate production. The feed contains sources of both cysteine and tyrosine and should not be supplemented with any additional alkaline feed.

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

Reconstitution method to prepare 10L Cellvento® 4CHO medium

- Slowly add 237 grams of powder to 8.0 L of Milli-Q® or similar cell culture grade water at room temperature (25 °C) in an appropriately sized container
- Rinse medium container as necessary to remove remaining powder
- Allow to dissolve with vigorous mixing for 30 minutes (solution will still be slightly turbid)
- Add 2 g/L sodium bicarbonate and stir until dissolved (~10 minutes)
- Add cell culture grade water to reach a final volume of 10 L and stir until dissolved (~10 minutes)
  - Confirm a final pH of 7.0 ± 0.3
- Measure the osmolality of the solution. Final osmolality should be at 310 ± 30 mOsmol/kg
- Immediately filter using a sterilizing-grade filter (≤0.22 µm). For filter recommendations, see Page 3
- Store at 2-8 °C protected from light
  - Reconstituted Cellvento® 4CHO liquid medium is stable for at least 90 days
  - When supplements are added, the liquid media is stable for max 4 weeks

Note: This medium does NOT contain L-Glutamine or hypoxanthine and thymidine. Aseptically supplement as required prior to use.

Storage

- Dry powder and compacted medium should be stored at 2–8 °C protected from light.
- Do not use after expiration date.

Shelf life: 12 months

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Using Cellvento® 4CHO medium in fed-batch mode

- Add 4-8 mM L-Glutamine to Cellvento® 4CHO medium prior to use with non-GS CHO cells lines
- Add 1x HT prior to use with non-dhfr systems
- Supplementation with a surfactant (e.g. poloxamer) is not required to use this product
- Cell selection agents should be added as required during the seed train expansion. In general, we recommend removing the selective pressure during the fed-batch production step and culture

Direct media adaptation

Cell lines may be adapted directly into Cellvento® 4CHO 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 any of the first generation Cellvento® product can be directly thawed or cultured in Cellvento® 4CHO.

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® 4CHO medium (in %)	Seeding density ($x10^5$ 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® 4CHO medium and cell culture grade 10% dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento® 4CHO 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 $x10^6$ cells/mL and viability >95%
- Centrifuge at 1200-1500 rpm for 5 min (200-300 g)
- Discard the supernatant and re-suspend cells in cold freezing medium at $1x10^7$ - $2x10^7$ viable cells/mL, and transfer the cell suspension into sterile cryovials with 1 mL each vial
- Freezing procedure with a freezing container containing isopropanol: Place the cryovials into the cryobox, and freeze the cells following the sequential procedure with decreasing temperatures:
  - 30 min at 4 °C
  - 2-4 hrs at -20 °C
  - over night 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 4 °C 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 1200-1500 rpm for 5 min
- Discard the supernatant, re-suspend the cells in fresh culture medium (Cellvento® 4CHO medium) in order to achieve a seeding density of $3x10^5$ – $5x10^5$ cells/mL, and transfer to a 125 mL Erlenmeyer flask for cultivation. Culture the cells in a 37 °C CO_2 incubator with 5% CO_2, 80% humidity and a ≥1 $x10^6$ cells/mL. Thereafter, sub-culture following standard protocols

Ordering information for Cellvento® 4CHO

Catalogue number	Product name	Pkg. size	Equivalent
1.03795.0010	Cellvento® 4CHO COMP	0.237 kg	10 liters
1.03795.0100	Cellvento® 4CHO COMP	2.370 kg	100 liters

Ordering information for Cellvento® 4Feed

Catalogue number	Product name	Pkg. size	Equivalent
1.03796.0005	Cellvento® 4Feed COMP	0.651 kg	5 liters
1.03796.0010	Cellvento® 4Feed COMP	1.304 kg	10 liters
1.03796.0050	Cellvento® 4Feed COMP	6.518 kg	50 liters

Ordering information for cell culture additives

Catalogue number	Product name	Pkg. size
1.37020.5000	Sodium hydroxid pellets suitable for the biopharmaceutical production EMPROVE® bio	5 kg
1.37013.2500	Sodium hydrogen carbonate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JP	2.5 kg
1.00286.1000	L-Glutamine suitable for use as excipient EMPROVE® exp DAB, USP	1 kg

Ordering information for Sterilizing-grade filters

	Bacteria Removal	Mycoplasma & Bacteria Removal	Virus, Mycoplasma & Bacteria
Volume (L)	**Millipore® Express SHC**	**Millipore® Express SHR with Prefilter**	**Viresolve® Barrier**
5	KHGES015FF3	KHVES015FF3	VBKG005TC1
10	KHGES015FF3	KHVES015FF3	VBKG015TC1
50	KHGES003FF3	KHVES006FF3	VBKG050TC1

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

To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645	Spain: 901 516 645 Option 1
Germany: 069 86798021	Switzerland: 0848 645 645
Italy: 848 845 645	United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840
Or visit: **MerckMillipore.com/offices**
For Technical Service visit: **MerckMillipore.com/techservice**

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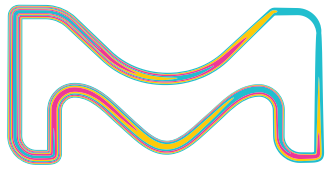

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