SAFC®
Pharma & Biopharma Raw Material Solutions

Cellvento® 4CHO-X

Expansion Medium

Product description

There are several advantages to using perfusion in your seed train, namely, flexibility in your production processes, increase in capacity and footprint reduction.

Reducing the number of bioreactors in your seed train leads to a better utilization and eliminates traditional bottlenecks (compressed seed train). Additionally, using N-1 perfusion enables the inoculation of a production bioreactor with higher cell densities for high seed production process formats.

Cellvento® 4CHO-X Expansion Medium has been specifically developed for N-1 perfusion for Chinese Hamster Ovary (CHO) cell lines. It is formulated with a high nutrient concentration to achieve low cell specific perfusion rates (CSPRs) at high cell densities. The medium is chemically defined, of non-animal origin, and contains no hydrolysates or components of unknown composition.

Application

Cellvento® 4CHO-X Expansion Medium has been specifically formulated to control the metabolic profile of the cells, both to minimize toxic byproducts and to ensure adequate nutrient levels for high cell densities. Preventing nutrient depletion can increase robustness and predictability of seed train operations. This medium supports high growth and viability, thus increasing cell biomass for cryopreservation or inoculation of another reactor. The medium addresses seed train intensification and shows a positive impact in IgG production, when combined in perfusion production with EX-CELL® Advanced HD Perfusion medium (24370C), and in Fed-batch production with e.g. Cellvento® 4CHO medium (103795) and Cellvento® 4Feed (103796). It is expected to work with other media platforms as well. Cellvento® 4CHO-X Expansion Medium can be used for all seed train expansion steps including N-1, reaching robust growth at low CSPRs.

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

Reconstitution method to prepare Cellvento® 4CHO-X Expansion Medium

- Measure 90 % of final required volume of Milli-Q® or similar cell culture grade water at room temperature (25 °C) in an appropriately sized container.
- Slowly add 26.2 g/L of medium, while stirring. Continue stirring for 30 minutes. Product will remain slightly turbid.
- Add 1.565 g/L sodium bicarbonate to the solution. Stir continuously for 30 minutes.
- Measure pH, which should be at 7.4 +/- 0.3. Adjust pH to cell line specific optimum using 5N HCl if desired.
- Add Milli-Q® or similar cell culture grade water to 100% final volume.
- Measure osmolality. Final osmolality should be at 310-370 mOsmol/kg.
- Immediately filter using a sterilizing-grade filter (≤0.22µm). For filter recommendations, see Page 3.
- Store product at 2-8 °C, protected from light until use.
  - reconstituted Cellvento® 4CHO-X Expansion Medium is stable for at least 90 days

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– 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 or add during powder addition.

- Dry powder and compacted medium should be stored at 2–8 °C protected from light.
- Do not use after expiration date.
- Shelf life: as reported on CoA

Using Cellvento® 4CHO-X medium in expansion and N-1 perfusion

Add 4-8 mM L-Glutamine to Cellvento® 4CHO-X Expansion 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 during the seed train expansion.

For using Cellvento® 4CHO-X Expansion Medium for perfusion, hydrate appropriate amount of medium according to hydration instructions and perfuse medium using appropriate perfusion equipment through the bioreactor. Monitoring of glucose concentration is recommended to adjust perfusion rate.

Direct media adaptation

Cell lines may be adapted directly into Cellvento® 4CHO-X Expansion 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.

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.

Cryopreservation

Viable cell banks may be created by freezing cells in 92.5 % Cellvento® 4CHO-X Expansion Medium and cell culture grade 7.5 % dimethyl sulfoxide (DMSO).

Cell freezing operation procedure:

- Mix sterile DMSO and Cellvento® 4CHO-X Expansion Medium 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), ideally at 4°C.
- Discard the supernatant and re-suspend cells in cold (4°C) freezing medium at $1x10^7$ - $2x10^7$ viable cells/mL, and transfer the cell suspension into sterile cryovials with 1 mL each vial
- Freeze vials directly at –80 °C over night and transfer and store the vials in the liquid nitrogen tank for long term storage
- Alternatively use a 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.

Ratio of current media vs Cellvento® 4CHO-X Expansion Medium (in %)	Seeding density ($x10^5$ cells/mL)	Evaluation of cell growh	Acceptance criteria for next step
75:25	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viabilty>80% over at least 2 passages
50:50	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viabilty>80% over at least 2 passages
25:75	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viabilty>80% over at least 2 passages
10:90	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; Viabilty>80% over at least 2 passages
0:100	3.0	Cell density, viability in mid-log growth phase	Adaptaion complete when cells maintaian normal doubling time; Viabilty>90% over at least 2 passages

Cell thawing and recovery procedure

- Prepare a water bath at 37 °C for cell thawing
- In a 125 mL Erlenmeyer flask, prepare 29 mL Cellvento® 4CHO-X Expansion 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) and the remaining ice has the approximate size of a rice grain.
- Transfer the CHO cell suspension from the cryovial to the 125 mL Erlenmeyer flask 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 vs. Cellvento® 4CHO-X Expansion Medium (in %)

In case DMSO removal is desired:

- 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) and the remaining ice has the approximate size of a rice grain
- 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-X 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 vs. Cellvento® 4CHO-X Expansion Medium (in %)

Subculturing

- Pre-warm hydrated Cellvento® 4CHO-X Expansion Medium to room temperature
- Aseptically remove a small volume of cell culture sample from the flask and count by trypan blue exclusion using a hematocytometer or an automated cell counter. Viability should be higher than 90% at all times
- Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of 2 – $3×10^5$ viable cells/mL for the targeted working volume
- Aseptically add the corresponding amount of fresh media pre-warmed at room temperature to the new flasks followed by the calculated amount of cells
- Incubate at 37 °C in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform (19 mm diameter orbit) rotating at 120-140 rpm
- Passage cells by repeating the above steps at least twice a week, and always maintaining the cells in exponential growth

Ordering information for Cellvento® 4CHO-X Expansion Medium

Cat. No.	Product name	Pkg. size	Equivalent
1.03840.0001	Cellvento® 4CHO-X COMP	0.026 kg	1 liter (sample size)
1.03840.0010	Cellvento® 4CHO-X COMP	0.262 kg	10 liters
1.03840.0100	Cellvento® 4CHO-X COMP	2.620 kg	100 liters
1.03840.0500	Cellvento® 4CHO-X COMP	13.098 kg	500 liters

Ordering information for cell culture additives

Cat. No.	Product name	Pkg. size
1.37020.5000	Sodium hydroxide 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
1.37117.0100	Dimethyl sulfoxide EMPROVE® Expert Ph EUR, USP	100 mL

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
1	KHGES015FF3	KHVES015FF3	VBKG005TC1
10	KHGES015FF3	KHVES015FF3	VBKG015TC1
100	KHGES003FF3	KHVES006FF3	VBKG050TC1

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

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To place an order or receive technical assistance

In the U.S. and Canada, call toll-free 1-800-645-5476
For other countries across Europe and the world, please visit: **EMDMillipore.com/offices**
For Technical Service, please visit: **EMDMillipore.com/techservice**

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