Product Information

1.01885 Cellvento™ CHO-200 Chemically defined cell culture medium

Product description

Cellvento™ CHO-200 is a chemically defined medium of non-animal origin for use in fed-batch culture. The product is intended for use in the development and manufacturing of bio-therapeutics in Chinese Hamster Ovary (CHO) cell-based expression systems.

Cellvento™ CHO-200 has been formulated without phenol red L-glutamine, hypoxanthine, thymidine, or sodium bicarbonate and is supplied in dry powder form.

Application

Cellvento™ CHO-200 has been designed for high growth and performance of CHO-S cells in fed-batch culture in combination with Cellvento™ Feed products, but may be suitable for use with other commonly used CHO cell lines.

After filtration ensure applying sterile techniques when handling or supplementing this medium. This product is intended for research or further manufacturing but not for human or therapeutic use.

Media preparation

Cellvento™ CHO-200 lacks glutamine in order to maximize flexibility for use. Prior to use with non-GS cell lines this medium should be supplemented with 4-8 mM L-glutamine (Art. No. 1.00286).

Similarly, standard concentrations of hypoxanthine and thymidine should be added to Cellvento™ CHO-200 for applications where non-DHFR (dihydrofolate reductase) amplified systems are used.

Supplementation with a surfactant (i.e. Pluronic) is not required to use Cellvento™ CHO-200.

For the media preparation sodium bicarbonate needs to be added (Art. No. 1.37013) following the instruction below.

Reconstitution method to generate 10 L Cellvento™ CHO-200 medium

1. Slowly add 232.5 grams of powder to 8.0 L of Milli-Q® or similar cell culture grade water in an appropriately sized container.
2. Allow to dissolve by gently stirring for 45–60 minutes (solution will still be slightly turbid). Then adjust pH to 5.5 +/– 0.2 using 5 M sodium hydroxide.
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 and confirm a final pH of 7.0 +/– 0.2.
6. Measure the osmolality of the solution. Final osmolality should be at 315 +/– 10 mOsmol/kg. Adjust with a 5 M NaCl solution if necessary.
7. Sterilize by membrane filtration using a 0.2 µm filter.
8. Store at 2–8 °C protected from light.

Specifications

Appearance	white to light pink
Solubility	well soluble
pH* (without supplement)	3.9–4.5
Osmolality* (without supplement)	240–290 mOsmol/kg
Water content	≤ 3.5 %
Endotoxin	≤ 1.0 I.U./mL
Microbiological purity (total viable aerobic count)	≤ 100 cfu/g
Mycoplasma	absent
Cell growth test	1 x 10^6 cells/mL after 72 hours

* Data for pH and osmolality are shown without bicarbonate supplementation.

Storage

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

Shelf life

12 months

Direct media adaptation of CHO cells*

Some cells can be directly adapted to Cellvento™ CHO-200 medium. Cells should be seeded at 2.5×10^5–3.5×10^5 cells/mL and then sub-cultured when densities reach 1×10^6–3×10^6 cells/mL and ≥ 90 % viability. Adaptation is complete when cells maintain normal doubling time and VCD ≥ 90 % over at least 2 passages.

* Cells cultured in Cellvento™ CHO-100 growth medium can often be sub-cultured directly into Cellvento™ CHO-200 medium. Cells banked in Cellvento™ CHO-100 should be thawed and maintained in Cellvento™ CHO-100 for at least 1 passage prior to sub-culturing in Cellvento™ CHO-200.

Sequential media adaptation of CHO cells

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.

Relation of current media vs. Cellvento™ CHO-200 (in %)	Seeding density ($x10^5$ cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
75:25	3.0–0.4	Cell density, viability in mid-log growth phase	Normal cell doubling time; VCD ≥ 90 % over at least 2 passages
50:50	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; VCD ≥ 90 % over at least 2 passages
25:75	3.0	Cell density, viability in mid-log growth phase	Normal cell doubling time; VCD ≥ 90 % 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; VCD ≥ 90 % over at least 2 passages

Cryopreservation

Viable cell banks may be created by freezing cells in 90 % Cellvento™ CHO-200 and 10 % Dimethyl sulfoxide (DMSO).

Freezing:

- Cultures should be in logarithmic phase and at least 90 % viable for cryopreservation.
- Prepare the freezing medium by combining Cellvento™ CHO-200 at 90 % with dimethyl sulfoxide (DMSO) at 10 %. Store at 2–8 °C until use.
- Precipitate cells by centrifugation at 1,000 rpm for 5 minutes (100–200 xg (rcf)).
- Decant supernatant then and re-suspend cells in cold freezing medium at 5×10^6–1×10^7 viable cells/mL.
- Transfer aliquots of the cell suspension into sterile cryovials.
- Freeze cells at –80 °C for 24 hours and then transfer to liquid nitrogen for long-term storage.

Thawing:

- Thaw a vial of frozen cells rapidly in a 37 °C water bath.
- Transfer cells to a centrifuge tube with 10 mL of Cellvento™ CHO-200 medium at room temperature.
- Precipitate cells by centrifugation at 1,000 rpm for 5 minutes.
- Decant supernatant and re-suspend cells in an adequate volume of Cellvento™ CHO-200 media. Seed a shaker flask at 3×10^5–5×10^5 cells/mL.
- Incubate cells at 37 °C and 5 % CO_2 until densities reach 1×10^6 cells/mL. Thereafter, sub-culture following standard protocols.

Ordering information Cellvento™ CHO-200

Catalog number	Product name	Pkg. size	Equivalent
1.01885.0010	Cellvento™ CHO-200	0.232 kg	10 liters
1.01885.0100	Cellvento™ CHO-200	2.325 kg	100 liters

For more information and documentation please contact:

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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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