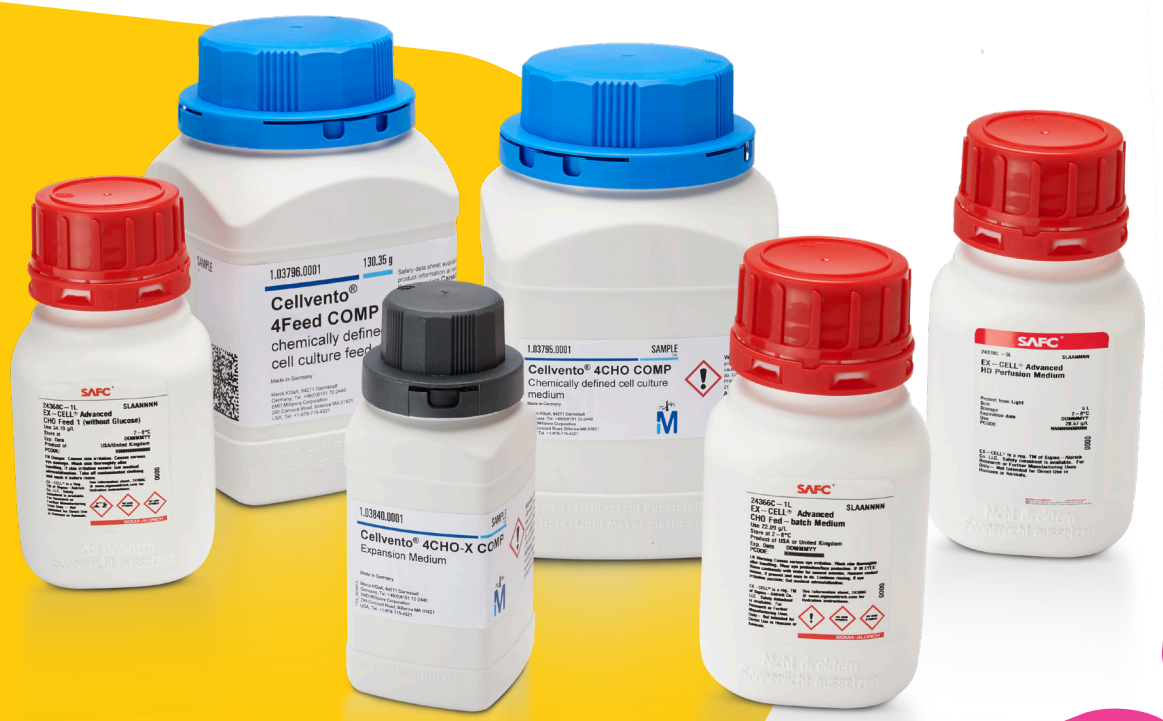
Cell Culture Media 4CHO Kit



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Pharma & Biopharma Raw Material Solutions

Cell Culture Media 4CHO Kit

Explore our platform offering for best success

Looking to improve your fed-batch process or consider perfusion as alternative?

- This medium kit supports evaluation from cell expansion to production in fed-batch or perfusion mode.
- The kit includes 6 samples for R&D evaluation purpose (non-GMP).
- Media and feeds are compatible.

Benefits

- Quick access to our compatible media platform.
- Formulations supporting diverse CHO cell lines from expansion to production scale.
- Expansion medium to optimally prepare the cells for production mode.
- Basal media formulations supporting different CHO cell line needs.
- High concentrated feed, neutral pH and simple hydration.
- Perfusion medium designed to support the nutrient requirements during continuous processing.

Contents

Media Selection Overview

Perfused Seed Train (N-1) & Cryopreservation

Cellvento® 4CHO-X Expansion Medium

Fed Batch

EX-CELL® Advanced Medium
Cellvento® 4CHO
+
EX-CELL® Advanced Feed
Cellvento® 4Feed

Perfusion

EX-CELL® Advanced HD Perfusion Medium

Perfused Seed Train

Cellvento® 4CHO-X Expansion Medium supports cell growth at low CSPR and is developed for perfused seed train application to spike high cell densities to the production bioreactor (N) to enhance performance in both fed-batch and perfusion. Suits as well as a cryomedium when supplemented with DMSO.

Fed-batch Applications

Basal media EX-CELL® Advanced & Cellvento® 4CHO can be combined with Cellvento® 4Feed or a Feed Mix. Cellvento® 4Feed offers the benefit of higher concentration and incorporation of modified amino acids.

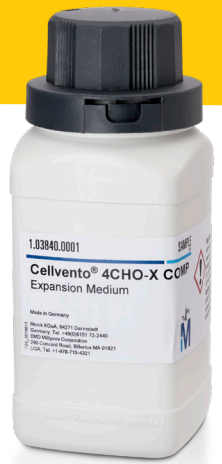
Perfusion Applications

EX-CELL® Advanced HD Perfusion Medium supports perfusion in production scale. This medium can reach and maintain high cell densities at low cell specific perfusion rates (CSPR), while supporting high volumetric productivities.

- **Recommended for all CHO cell lines**
- **Not recommended for CHOZN® cell line**

Media for Perfusion Processes

Reconstitution Protocol



Cellvento® 4CHO-X Expansion Medium

103840 – Cell Expansion Medium

1. Measure 0.9 L of Milli-Q® or similar cell culture grade water at room temperature (25 °C) in an appropriately sized container.
2. Slowly add 26.2 grams of medium, while stirring. Continue stirring for 30 minutes. Product will remain slightly turbid.
3. Add 1.565 g/L sodium bicarbonate to the solution. Stir continuously for 30 minutes.
4. Measure pH, which should be at pH 7.4 ± 0.3. Adjust pH to cell line specific optimum using 5N HCl if desired.
5. Add cell culture grade water to a final volume of 1 L.
6. Measure the osmolality. Final osmolality should be at 310-370 mOsmol/kg.
7. Immediately filter using a sterilizing-grade (≤0.22 µm) bottle cap or capsule filter.
   - Store at 2-8 °C protected from light.
   - Reconstituted liquid Cellvento® 4CHO-X Expansion Medium is stable for at least 90 days.
   - When supplements are added, the liquid medium is stable for max. 4 weeks.

Ex-CELL® Advanced HD Perfusion Medium

24370C - Perfusion Medium

1. Measure 0.8 L of Milli-Q® or similar cell culture grade water at room temperature (25 °C) in an appropriately sized container.
2. Slowly add 28.57 grams of medium, while stirring. Continue stirring for 15 minutes. Product will remain slightly turbid.
3. Measure pH and adjust to 9.1 ± 0.05 with 5N NaOH. Continue stirring for 10 minutes.
4. Adjust pH to 6.4 ± 0.1 using 5N HCl.
5. Add 1.565 g/L sodium bicarbonate to the solution and stir continuously for 15 minutes.
6. Add cell culture grade water to reach a final volume of 1 L.
7. Measure osmolality. Final osmolality should be at 320-360 mOsmol/kg.
8. Immediately filter using a sterilizing-grade (≤0.22 µm) bottle cap or capsule filter.
9. Store product at 2-8 °C protected from light.
   - Reconstituted medium is stable for at least 30 days.

Fed-Batch System 1

Reconstitution Protocol

Cellvento® 4CHO COMP

103795 - Production Medium

1. Slowly add 23.7 g of Medium to 0.8 L of Milli-Q® or similar cell culture grade water at room temperature (25 °C) in an appropriately sized container.
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 a final volume of 1 L. Confirm a final pH of 7.0 ± 0.3.
5. Measure the osmolality of the solution. Final osmolality should be at 310 ± 30 mOsmol/kg.
6. Immediately filter using a sterilizing-grade (≤0.22µm) bottle cap or capsule filter.
7. Store at 2-8°C protected from light.
   - Reconstituted liquid Cellvento® 4CHO medium is stable for at least 90 days.
   - When supplements are added, the liquid medium is stable for max 4 weeks.

Cellvento® 4Feed COMP

103796 - High concentrated Feed

1. Slow add 130.35 g Feed to 0.8L of Milli-Q® or similar cell culture water grade at room temperature in an appropriate sized container.
2. Mix vigorously for 45 min until fully dissolved.
3. Slowly add NaOH to adjust pH to 7.0 ± 0.3.
4. Add cell culture grade water to a final volume of 1L. Confirm final pH of 7.0 ± 0.3.
5. Measure osmolality of the solution. Final osmolality should be 1220 ± 50 mOsmol/kg. Immediately filter using a sterilizing-grade filter (≤0.22µm).
6. Store at 2-8 °C protected from light.
   - Reconstituted liquid Cellvento® 4Feed media is stable for 60 days.
   - When a bottle is opened, liquid feed is stable for max. 3 weeks.

Fed-batch System 1

Cellvento® 4CHO/4Feed Fed-batch

Experimental condition	Operating Parameter
Culture type	Spin tubes with vented cap
Initial working volume	30 mL
Inoculation density	2-3 x10^5 cells/mL
Agitation rate	320 rpm
Temperature	37.0 ± 0.5 °C
Incubator pCO2	5 %
Media pH	7.0 +/- 0.3
Harvest criterion	End culture when viability < 50-70 %
Sampling points	Study days 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
Feed volume	1 % – 6 % (v/v)
Feed schedule	48-72 hour feed intervals (See below for guidance)

Recommendation for Fed-batch evaluation

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.

Media supplementation

- 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.

Recommended Feeding Option

Initiate the feeding only when viable cell density is ≥ 2 x 10^6 cells/ml and no earlier than day 3 (to avoid over-feeding).

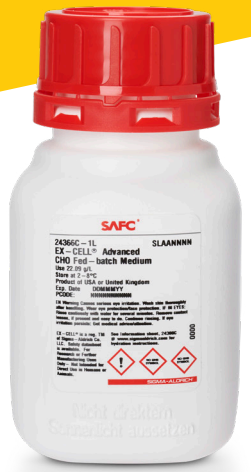
Maintain supplementation with feed supplements and glucose until culture viability is less than 80 %.

Terminate and harvest cultures when viability drops below 50-70 %.

Culture Day	Addition Order	1	2	3	4	5	6	7	8	9 or 10	11	12	13	14
Cellvento® 4Feed (%v/v)	1			3		3		6		3		3		
Glucose	2	Monitor daily and maintain at 4-6 g/L												

Fed-Batch System 2

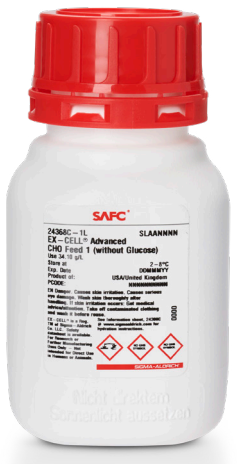
Reconstitution Protocol



EX-CELL® Advanced CHO Fed-batch Medium

24366C - Production Medium

1. Slowly add 22.09 g of powder to 0.8 L of Milli-Q® or similar cell culture grade water (ambient temperature).
2. Mix vigorously for 15 min.
3. Measure pH and adjust pH to 5.0, stir for 5 min.
4. Add 1.9 g of sodium bicarbonate. Stir for at least 30 min.
5. Adjust pH to 7.2 ± 0.1.
6. Add water to 1 L. Confirm pH is 7.2 ± 0.1.
7. Measure osmolality (Specification: 280-320 mOsmol/kg).
8. Sterile filter the medium using a 0.22 µm low protein binding filter.
9. Store at 2-8 °C protected from light.
    - Reconstituted medium is stable for 180 days after preparation (unopened) and stable for 30 days AFTER opening.



EX-CELL® Advanced Feed 1

24368C - Companion Feed

1. Measure approximately 0.8 L of of Milli-Q® or similar cell culture grade water (recommended water temperature is 25 °C to 40 °C).
2. Slowly add 34.1 g feed, while stirring.
3. Continually stir for 30 minutes. Product will remain slightly turbid.
4. Adjust pH to 9.5 ± 0.1. Continue mixing for 10 minutes. Product will be clear.
5. Lower pH to 8.5 using 5N HCl. Continue mixing for 10 minutes.
6. Add cell culture grade water to a final volume of 1 L.
7. Immediately sterile filter with low protein binding filter membrane. (<0.22 µm).
8. Store feed at 2-8 °C in the dark until use. Discard any unused feed after one month.

Fed-batch System 2

EX-CELL® Advanced Fed-batch

Experimental condition	Operating Parameter
Culture type	Shake flasks (E125)
Initial working volume	30 mL
Inoculation density	5-10 x10^5 cells/mL
Agitation rate	Own cell culture agitation rate
Temperature	37.0 ± 0.5 °C
Incubator pCO2	5 %
Media pH	7.2 +/- 0.3
Harvest criterion	End culture when viability < 70 %
Sampling points	Study days 3, 5, 7, 9, 11, 13, 14
Feed volume	5 % – 10 % (v/v)
Feed schedule	48-72 hour feed intervals (See below for guidance)

Recommendation for fed-batch evaluation

Usage Instructions:

Please add before use:

- L-glutamine (2-8 mM) for applications not using Glutamine Synthetase (GS) selection.
- HT supplement for applications not requiring Dihydrofolate Reductase (DHFR) selection.

Initiating cultures:

Thaw cell line according to own laboratory instructions, using pre-warmed medium. Seed at cell densities 0.5 – 1.0 x10^6 cells/mL for first two passages before returning to normal maintenance cell densities.

Subculturing/Adaptation:

Subculturing - Ensure < 25% final volume carry over in the first few subcultures. Centrifuge if >25% final volume. *Adaptation* – perform step-wise medium adaptation at 25/75, 50/50, 75/25, 100 % with current and EXCELL® Advanced medium. Perform at least 3-5 passages per adaptation ensuring VCD > 1.0 x10^6 cells/mL and Viability >90 % before progressing.

Cryopreservation:

Freeze desired quantity of cells in 46.5 % cold medium, 46.5 % conditioned medium, 7 % DMSO. Freeze down using standard freezing procedures.

Culture Day	Addition Order	1	2	3	4	5	6	7	8	9 or 10	11	12	13	14
EXCELL® Advanced Feed 1 (%v/v)	1			5		5		10		7.5		7.5		
Glucose	2	Monitor daily and maintain at >2 g/L												

Feed Mixing Protocol

Cellvento® 4Feed / EX-CELL® Advanced Feed

Mixing Procedure and Recommendations

As cell lines can inherently be diverse, it can be recommended when evaluating media to try several different mix levels to find the optimal level of feed for your process.

Recommended Mixing Levels

EX-CELL® Advanced Feed is best utilized as a feed supplement in conjunction with the Cellvento® 4Feed. However, Cellvento® 4Feed may offer the optimal performance output with utilizing EX-CELL® Advanced Feed as a feed supplement. We recommend to evaluate Cellvento® 4Feed both as a standalone feed and as a mixWhen supplements are added, the liquid medium is stable for max 4 weeks.

Reconstitute the feeds according to their individual protocols

Method for 50:50 volume blend of 2 L of Medium

1. Add 1 L of reconstituted EX-CELL® Advanced Feed (24368C) to mixer.
2. Add 1 L of reconstituted Cellvento® 4Feed (1.03796) to same mixer.
3. Mix together for 10 minutes until homogenous.
4. Measure pH (informational).
5. Measure Osmolality (informational).
6. Immediately sterile filter with low protein binding filter membrane. (<0.22 microns).
7. Store feed at 2-8 °C in the dark until use. Discard any unused feed after one month.

Mix Type	Cellvento® 4Feed (1.03796) per 1 L	EX-CELL® Advanced Feed (24368C) per 1 L	Estimated Concentration (g/L)
100	1 L	0 L	130
75:25	750 mL	250 mL	106
50:50	500 mL	500 mL	82
25:75	250 mL	750 mL	58

The following feed schedule is recommended for use for all the mixes noted above:

Percentages are based on the working volume of the bioreactor at the time of feeding, not the initial volume.

NOTE: There are few points to consider when mixing. At scale these media are only available as separate dry powder media and will still need to be ordered and reconstituted separately which includes sterile filtering for both. They can be combined as liquids prior to being added to the bioreactor and thus can sustain a one feed approach.

TAKE THE RIGHT PATH UPSTREAM

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- Maximize Productivity
- Meet Quality Requirements
- Simplify Operations
- Scale Up
- Prevent Contaminants

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