Application note

CHO Prime media and feed platform

The best option for your cell culture

CHO Prime media products

CHO Prime Medium w.o. HT

CHO Prime LG Medium w.o. HT

CHO Prime feed products

CHO Prime Feed A

CHO Prime Feed B

All CHO Prime products are suitable for laboratory use and manufacturing.

Product description

CHO Prime products are chemically defined (CD), protein-free, and specifically optimised for recombinant protein expression in Chinese Hamster Ovary (CHO) cells. Manufactured under GMP-compliant conditions, all formulations are free from animal and human-derived components (ADCF), ensuring the highest safety and regulatory standards.

Engineered to enhance productivity in bioprocessing, CHO Prime products support maximised yield and consistent product quality. By selecting the appropriate formulation and optimising feed strategies, processes can be tailored to meet specific performance goals. All products are suitable for biomanufacturing with CHO-GS, CHO-K1, CHO-S and CHO-DG44 cell lines. All products are HT-free (without hypoxanthine and thymidine) and are ideal for cell bank production and routine cultivation. After addition of HT all products can be used for productivity screenings and commercial manufacturing. Comprehensive technical and scientific support is available to help you maximise process efficiency and ensure consistent, high-quality biologic production.

Products and storage

Product	Catalog number	Package size	Storage	Shelf life
CHO Prime Medium w.o. HT	Liquid: C250006S	1 L	2-8°C protect from light	t.b.d*
	Powder: C250001X	5 L 100 L 500 L	2-8°C, protect from light, dry environment	9 months for powder*
CHO Prime LG Medium w.o. HT	Liquid: C250007R	1 L	2-8°C, protect from light	t.b.d*
	Powder: C250002W	5 L 100 L 500 L	2-8°C, protect from light, dry environment	9 months for powder *
CHO Prime Feed A	Liquid: C250008Q	0.5 L	2-8°C, protect from light	12 month
	Powder: C250003V	25 L	2-8°C, protect from light, dry environment	24 months for powder, 12 month after hydration
CHO Prime Feed B	Liquid: C250009Z	0.5 L	2-8°C, protect from light	12 months
	Powder: C250004U	25 L	2-8°C, protect from light, dry environment	24 months for powder, 12 months after hydration

* Stability studies are ongoing for a targeted shelf life of 36 months for powder products and data is continuously updated. Current expiry date can be provided on request or please refer to the product label.

Culture conditions

It is recommended to culture cells in CHO Prime media under an 8% CO_2 atmosphere to ensure optimal growth conditions. To maintain cells in the logarithmic growth phase, regular passaging every three to four days is advised.

Preparation of complete growth medium

All CHO Prime media are free of L-glutamine but contain glutamine derivatives. They are not containing hypoxanthine and thymidine (HT). Prior to use, supplement the medium with L-glutamine to a final concentration of 2–8 mM. For applications that do not require DHFR amplification – or to maximise productivity, such as in fed-batch processes – it is recommended to add HT supplement.

Glucose supplementation is only necessary for batch or fed-batch cultivation; for routine passaging, no additional glucose is required. Depending on the clumping tendency of the cell line, the addition of an anti-clumping agent can be beneficial in fed-batch cultivations.

Recovery of frozen cells (starting protocol)

Rapidly thaw frozen cells at 37 °C. The thawing process should not exceed 10 minutes. Carefully monitor the vial during thawing and remove it promptly once only a small ice crystal remains. Disinfect the vial with 70 % ethanol and transfer it into a laminar flow hood.

Gently transfer the contents into a centrifuge tube containing 9 mL of pre-warmed medium. Centrifuge at 200 *g* for 5 minutes at room temperature. Discard the supernatant and resuspend the cell pellet in 5–8 mL of pre-warmed complete growth medium.

Transfer the resuspended cells into a 125 mL shake flask containing 20–30 mL of growth medium. An initial cell density of $3–5 \times 10^5$ viable cells/mL and a shaking speed of 120–150 rpm are recommended. Cell viability and density should be monitored regularly.

Note: The protocol may require optimisation for cell lines sensitive to shear stress, or when higher initial cell densities or alternative shaking speeds are required.

Subculturing of cells

Regular passaging is essential to maintain cells in the logarithmic growth phase. It is recommended to subculture the cells every 3 to 4 days, using one of the following methods:

Option 1: Pellet the cells by centrifugation at 200 *g* for 5 minutes at room temperature, then resuspend the pellet in a fresh, complete growth medium.

Option 2: Dilute the existing cell suspension directly with fresh, complete growth medium. For this approach, a minimum dilution ratio of 1:3 (parts cell suspension: parts fresh medium) is recommended. If the required volume of cell culture for the next inoculation exceeds 33 % of the total culture volume, option 1 is recommended.

A seeding density of $0.2–1.2 \times 10^6$ viable cells/mL is advised. This can be adjusted depending on the desired inoculation density for (fed-)batch processes. Cell viability and density should be monitored regularly.

Adaptation of CHO cells to CHO Prime media

CHO cell lines, which were not initially generated in CHO Prime media can be adapted using a sequential adaptation strategy.

Option 1: Adherent Cell Lines (FBS-dependent)

Adherent cell lines can be adapted by gradually increasing the proportion of CHO Prime Medium, while reducing the amount of the original FBS-supplemented medium. For example, the proportion of CHO Prime Medium can be increased every second or third passage as follows:

10% → 20% → 50% → 75% → 90% → 95% → 100%.

Cell viability and density should be monitored regularly throughout the process. Once the cells begin to detach, initiate gentle shaking at a low speed (e.g. 50 rpm). The shaking speed can be increased once the doubling time drops below 30 hours.

Option 2: Suspension Cell Lines (FBS-independent)

Suspension cell lines can generally be adapted to CHO Prime Medium more rapidly than adherent lines. However, direct adaptation is not recommended. As an example, the proportion of CHO Prime Medium may be increased every second or third passage in the following steps:

25% → 50% → 75% → 90% → 100%.

Cell viability and density should be monitored regularly. Extended cultivation over several weeks may further reduce the doubling time, provided the cell line remains stable during prolonged culture.

Note: Adaptation may take several weeks, and protocol adjustments may be required depending on the specific cell line. Comprehensive technical and scientific support is available to help optimise the adaptation process.

Cryopreservation of Cells Using CHO Prime Media

CHO Prime media are fully suitable for cryopreservation. A freezing medium can be prepared by adding 7.5 - 10 % DMSO to either the basal medium or the supplemented complete growth medium. It is recommended to store DMSO-containing freezing medium at 2–8 °C prior to use and to prepare it freshly each time.

Cells should be frozen during the logarithmic growth phase, with a viability above 90 %. Harvest the appropriate volume of cell suspension by centrifugation (e.g. 200 *g* for 5 minutes). Remove the

supernatant and gently resuspend the cell pellet in cold freezing medium, then quickly dispense into cryogenic vials.

A freezing rate of 1 °C per minute is recommended. This can be achieved using either a suitable freezing container or a controlled-rate freezer. For long-term storage, vials should be transferred to liquid nitrogen.

Note: A freezing density of $1–5 \times 10^7$ cells/mL is recommended. However, depending on the cell line, lower or higher densities — as well as adjustments of the DMSO concentration — may be beneficial and can be evaluated accordingly.

Application of CHO Prime products in Fed-batch cultivation

CHO Prime products are suitable for routine cultivation, cell banking, seed train expansion, and high-performing batch and fed-batch applications.

After thawing, at least 3 - 4 passages are recommended before inoculating a batch or fed-batch process. During seed train expansion, the inoculation density can be increased to reach the desired cell number for production-scale inoculation. Additional supplements may be required to maximise productivity (please refer to "Preparation of complete growth medium").

For fed-batch processes, CHO Prime feeds must be used in combination with CHO Prime media. CHO Prime Feed A and CHO Prime Feed B are highly concentrated nutrient formulations designed to enhance cellular growth and product yield. Both feeds are intended to be used together in a 10:1 ratio. Additionally, it is recommended to add an initial amount of 2.6-5.2 mL/L of CHO Prime Feed B to the culture on the inoculation day of the fed-batch.

Exemplary feeding strategy: If a daily feed rate of 1 % (v/v) CHO Prime Feed A (based on the initial culture volume) is selected, it should be combined with 0.1 % (v/v) CHO Prime Feed B per day.

Note: The total feed volume and the feeding strategy must be optimised based on process-specific parameters. For example, higher inoculation densities require increased feed rates. Cell lines reaching higher peak cell densities typically demand more nutrients than those with lower densities. Examples of feed rates and process configurations are provided in the table below. However, the optimal feed rate should always be determined experimentally for each cell line and process.

It is recommended to initiate feeding on day 2 or 3 after fed-batch inoculation using both CHO Prime Feed A and CHO Prime Feed B. Although CHO Prime Feed A contains glucose, it may not fully meet the culture's daily glucose demand. Therefore, glucose levels should be monitored throughout the process

and maintained within a range of 2–8 g/L by supplementing as needed. CHO Prime products are also suitable for fed-batch processes with temperature shifts or reduced temperatures as well as perfusion and continuous manufacturing applications.

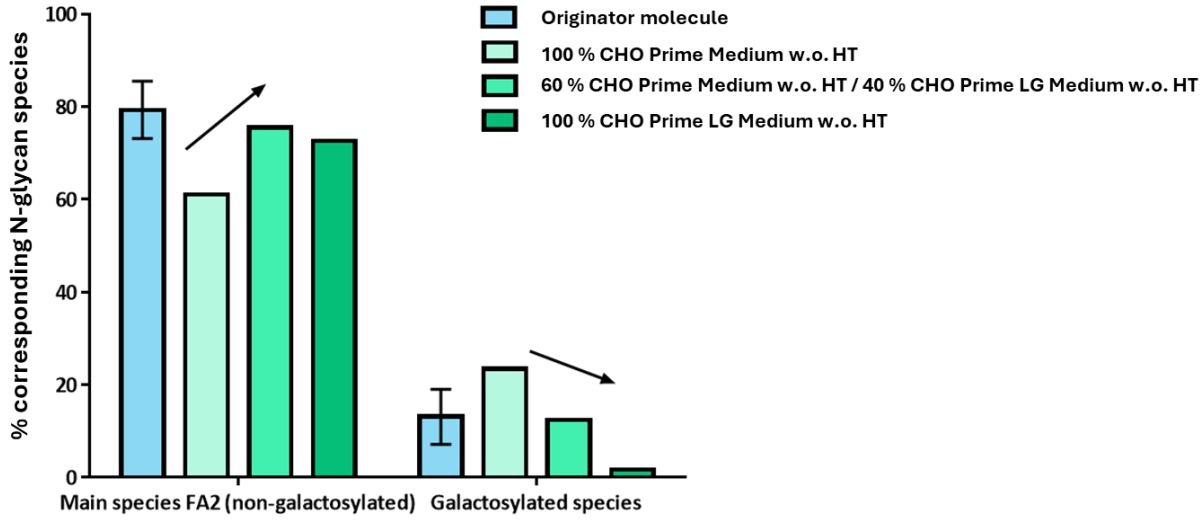
Scenario	Recommended feed rate of CHO Prime Feed A	Recommended feed rate of CHO Prime Feed B
Cell line A was inoculated at 3×10^5 cells/mL and/or grows to low peak cell densities (5×10^6 cells/mL) by day 7.	1.0 % (v/v of initial culture volume per day), starting on day 3. Test feed rates of 0.5 % and 1.5 % as well.	0.1 % (v/v of initial culture volume per day), starting on day 3. Test feed rates of 0.05 % and 0.15 % as well. Add 2.6 mL/L already on the inoculation day.
Cell line B was inoculated at 1.2×10^6 cells/mL and/or grows to high peak cell densities (5×10^7 cells/mL) by day 10.	2.5 % (v/v of initial culture volume per day), starting on day 2. Test feed rates of 2.0 % and 3.0 % as well.	2.5 % (v/v of initial culture volume per day), starting on day 2. Test feed rates of 2.0 % and 3.0 % as well. Add 5.2 mL/L already on the inoculation day.
Cell line C was inoculated at 1.2×10^6 cells/mL and/or grows to intermediate peak cell densities (2×10^7 cells/mL) by day 12.	2.0 % (v/v of initial culture volume per day), starting on day 2. Test feed rates of 1.5 % and 2.5 % as well.	2.0 % (v/v of initial culture volume per day), starting on day 2. Test feed rates of 1.5 % and 2.5 % as well. Add 2.6 mL/L already on the inoculation day.

Note: A fixed feed rate is generally recommended; however, a dynamic feeding strategy can also be advantageous, particularly for cell lines exhibiting a prolonged lag phase followed by exponential growth later in the fed-batch process. In such cases, it may be beneficial to increase the feed rate accordingly in the late exponential growth phase.

Application of CHO Prime LG Medium w.o. HT

CHO Prime LG Medium w.o. HT was developed to reduce galactosylated N-glycan species in manufacturing processes. A matching glycan profile is of particular interest in the context of biosimilar development. The medium can be used on its own or blended with CHO Prime Medium w.o. HT to modulate galactosylation to the desired level. The optimal blending ratio can be determined by combining the media in varying proportions and analysing the resulting product characteristics. An example of the expected outcome from a case study on biosimilar development is shown below.

Note: CHO Prime LG Medium w.o. HT requires appropriate supplementation (please refer to "Preparation of complete growth medium"), including HT supplement, L-glutamine, and anti-clumping agent as needed. Prior adaptation to this medium may be beneficial. Please refer to "Adaptation of CHO cells to CHO Prime media", Option 2, for more details. CHO Prime LG Medium does not significantly affect cellular growth, productivity, or other product quality parameters.



For further questions or any technical or scientific support, please contact us:

E-mail: info@ugabiopharma.com
Web: www.ugabiopharma.com
Phone: +49 3302 / 2024900

UGA Biopharma GmbH
Neuendorfstraße 20a
16761 Hennigsdorf
Germany