Application note

First CHOice® media and feed platform

The right CHOice for your cell culture

First CHOice® media products

First CHOice® Medium	**Plus** - New generation available!
First CHOice® Medium w.o. HT	➢ First CHOice® Medium Plus w.o. HT
First CHOice® LG Medium w.o. HT	➢ First CHOice® LG Medium Plus w.o. HT

First CHOice® feed products

First CHOice® Feed Alpha	➢ First CHOice® Feed Alpha Plus
First CHOice® Feed Beta	

All First CHOice® products are suitable for laboratory use and manufacturing.

Product description

First CHOice® products are chemically defined (CD) and protein-free, optimized for recombinant protein expression with Chinese Hamster Ovary (CHO) cells. All products are produced under GMP compliant conditions.

All First CHOice® products are animal and human component free (ADCF) and designed to boost productivity during bioprocessing. By adjusting the feed rate and selection of the right First CHOice® products, a maximum productivity and optimal product quality can be achieved. All products are suitable for biomanufacturing of biologic products using CHO-GS, CHO-K1, CHO-S or CHO-DG44.

First CHOice® Plus products are developed especially for high-productive cell lines growing to high peak cell densities and requiring a high supply of appropriate nutrients. Products without hypoxanthine and thymidine (HT) can be applied during cell bank manufacturing or routine cultivation. For productivity screenings or manufacturing, products with hypoxanthine and thymidine (HT) are available.

Close technical and scientific support is offered to maximize the process yield and product quality.

Products and storage

Product	Catalog number	Package size	Storage	Shelf life
First CHOice® Medium	Powder: F180004U	10 L 100 L 500 L	2-8°C, protect from light, dry environment	24 months for powder, 12 months after hydration
First CHOice® Medium w.o. HT	Liquid: F170012U	1 L	2-8°C protect from light	12 months
	Powder: F180015Q	10 L 100 L	2-8°C, protect from light, dry environment	24 months for powder, 12 months after hydration
First CHOice® Medium Plus w.o. HT New	Liquid: F250001X	1 L	2-8°C, protect from light	Expiry date on request*
	Powder: F240001Y	5 L 100 L 500 L	2-8°C, protect from light, dry environment	Expiry date on request*
First CHOice® LG Medium w.o. HT	Liquid: F220005W	1 L	2-8°C, protect from light	12 months
	Powder: F220003Y	10 L 100 L	2-8°C, protect from light, dry environment	18 months for powder*, 12 months after hydration
First CHOice® LG Medium Plus w.o. HT New	Liquid: F250002W	1 L	2-8°C, protect from light	Expiry date on request*
	Powder: F240002X	5 L 100 L 500 L	2-8°C, protect from light, dry environment	Expiry date on request*
First CHOice® Feed Alpha	Liquid: F180016Z	0.5 L	2-8°C, protect from light	4 weeks
	Powder: F180002W	5 L 25 L 100 L	2-8°C, protect from light, dry environment	24 months for powder, 4 weeks after hydration
First CHOice® Feed Alpha Plus New	Liquid: F220004X	0.5 L	2-8°C, protect from light	12 months
	Powder: F220002Z	5 L 25 L	2-8°C, protect from light, dry environment	24 months for powder, 12 months after hydration
First CHOice® Feed Beta	Liquid: F180017Y	0.2 L	2-8°C, protect from light	6 months
	Powder: F180014R	5 L 25 L 100 L	2-8°C, protect from light, dry environment	24 months for powder, 6 months after hydration

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

Culture conditions

For all First CHOice® products, cultivation in an 8% CO_2 environment is recommended. Cells should be maintained in the logarithmic growth phase through regular passaging, typically every three to four days.

Preparation of complete growth medium

All First CHOice® media are free from L-glutamine but contain its derivatives and are available with or without hypoxanthine and thymidine. Supplement the medium with L-glutamine to a final concentration of 2–8 mM prior to use. For applications not requiring DHFR amplification, or to achieve maximum productivity (e.g. in fed-batch cultivations), add HT supplement to a final concentration of 1x.

Glucose supplementation is only required in batch or fed-batch cultivation, whereas for routine passaging additional glucose is not necessary. Depending on the clumping tendency of the cell line, an anti-clumping agent may be required in fed-batch cultivations.

Recovery of frozen cells (starting protocol)

Rapidly thaw frozen cells at 37 °C. Thawing of the vials should not take longer than 10 minutes. Carefully monitor the thawing process and promptly remove the cryogenic vial once only a small ice crystal remains. Wipe it briefly with 70 % Ethanol and place it under the laminar flow hood. Transfer the content to 9 mL pre-warmed medium into a centrifugation tube. 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 resuspended cells into a 125 mL shake flask, containing 20 - 30 mL growth medium. An initial cell density of 3 - 5 x 10^5 viable cells/mL and 120 - 150 rpm is recommended. Regularly monitor cell viability and cell density.

Note: The protocol may require further adjustment if a cell line is sensitive to shear stress, or if higher initial cell densities or different shaking speeds are needed.

Subculturing of cells

Regular passaging is required to maintain the cells in a logarithmic growth phase. It is recommended to subculture the cells every 3 to 4 days either by pelleting the cells at 200 *g* for 5 minutes at room temperature and a subsequent resuspension in fresh complete growth medium (option 1) or by diluting the cell suspension with fresh complete growth medium (option 2). For the second option, at least a ratio of 1:3 (parts cell suspension / parts fresh medium) is recommended. If the required volume of cell culture for the inoculation exceeds 33 % of the total culture volume, then option 1 is recommended. A seeding

density of 0.2 - 1.2 x 10^6 viable cells/mL is recommended. The seeding density can be adapted depending on the targeted (fed-)batch inoculation density. Cell viability and cell density should be monitored regularly.

Adaptation of CHO cells to First CHOice® media

CHO cell lines not initially generated in First CHOice® media can be adapted through sequential adaptation.

Option 1: Adherent cells lines (FBS-dependent)

Adherent cell lines can be adapted by gradually increasing the proportion of First CHOice® Medium while reducing the proportion of the original FBS-supplemented medium. For example, the proportion of First CHOice® Medium may be increased every second or third passage from 10% to 20%, 50%, 75%, 90%, 95% and finally 100%. Cell viability and density should be monitored regularly. Once the cells begin to detach, start gentle shaking at a low speed (e.g. 50 rpm). Increase shaking speed once the doubling time decreases below 30 hours.

Option 2: Suspension cell lines (FBS-independent)

Suspension cell lines can generally be adapted to First CHOice® Medium more rapidly than adherent cell lines. However, direct adaptation is not recommended. As an example, the proportion of First CHOice® Medium may be increased every second or third passage from 25% to 50%, 75%, 90% and finally 100%. Cell viability and density should be monitored regularly. Extended cultivation over several weeks may further reduce doubling time, provided that the stability of the cell line allows it.

Note: Adaptation may take several weeks and protocols may require adjustments. A close technical and scientific support is offered to obtain an optimal outcome.

Cryopreservation

First CHOice® media can be used for cryopreservation without any concerns. Prepare a freezing medium by adding 7.5 - 10 % DMSO to either pure medium or supplemented complete growth medium. It is recommended to store DMSO-containing freezing medium at 2 - 8 °C prior to use and prepare it fresh every time. Cells should be frozen during the logarithmic growth phase, with viability exceeding 90%. An appropriate volume of cell suspension is then centrifuged (e.g. 200 *g* for 5 minutes). Remove the supernatant, resuspend the cell pellet in cold freezing medium and dispense quickly into cryogenic vials. A freezing rate of 1 °C/min is recommended either by using an adequate freezing container or a controlled rate freezer. For a long-term storage, vials should be stored in liquid nitrogen.

Note: A freezing density of 1 - 5 x 10^7 cells/mL is recommended. However, lower or higher densities can also be investigated and applied. The DMSO concentration may also need to be adjusted depending on the cell line.

Fed-batch cultivation using First CHOice® products

First CHOice® products can be used in routine cultivation, cell banking and seed trains as well as in high-performing batch and fed-batch applications. If frozen cells are recovered, at least 3 - 4 passages are recommended before inoculating a batch or fed-batch cultivation. During the seed train, the inoculation density can be increased to reach the desired cell number for the inoculation. Further supplements might be required to achieve the maximum productivity (please refer to "Preparation of complete growth medium").

For fed-batch cultivations, First CHOice® feeds need to be combined with First CHOice® media. First CHOice® Feed Alpha/ Feed Alpha Plus and First CHOice® Feed Beta are highly concentrated and contain different nutrients that help to increase cellular growth and product yield. Both feeds are intended to be used together in a ratio of 10 to 1. If, for example, a feed rate of 1 % First CHOice® Feed Alpha Plus per day (v/v of initial culture volume) is selected, it should be combined with a feed rate of 0.1 % First CHOice® Feed Beta per day (v/v of initial culture volume).

Note: The total amount of feeds added (feed rate) and the specific feeding regime needs to be adjusted based on various factors. For example, an increased inoculation density requires an increased feeding rate, or a cell line that grows to higher peak cell densities needs to receive higher feeding rates than a cell line with lower peak cell densities. Examples are given in the table below. However, an optimal feed rate should be determined experimentally for each specific cell line and process. It is recommended to start feeding of First CHOice® Feed Alpha/ First CHOice® Feed Alpha Plus and First CHOice® Feed Beta on day 3 after fed-batch inoculation. First CHOice® Feed Alpha/ First CHOice® Feed Alpha Plus contain glucose but may not be sufficient to cover the daily consumption. Monitor glucose throughout the process and maintain a level of 2 - 8 g/L by adding glucose as necessary.

First CHOice® products are also suitable for fed-batch processes requiring a temperature shift or reduced temperature as well as for perfusion or continuous manufacturing.

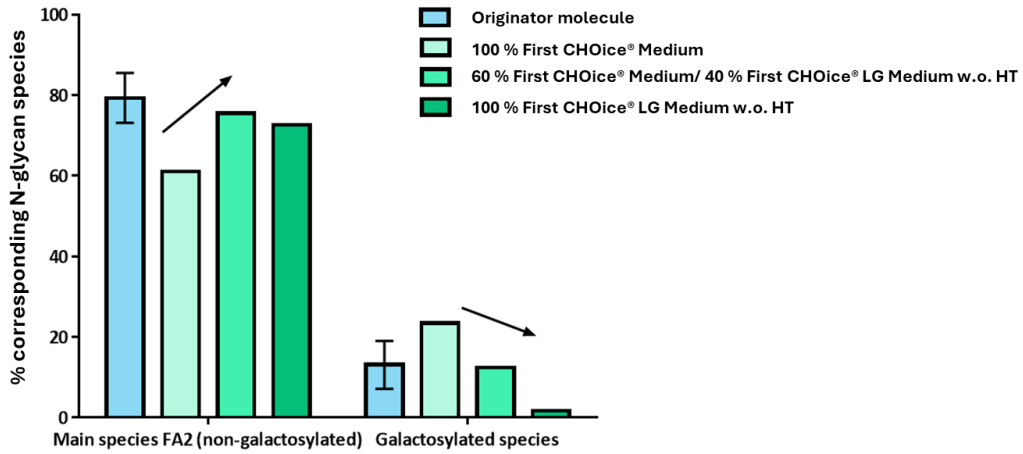
Scenario	Recommended feed rate of First CHOice® Feed Alpha/ Feed Alpha Plus	Recommended feed rate of First CHOice® Feed Beta
Cell line A is growing to low peak cell densities of 5 x 10^6 cells/mL, reached on day 7 and was inoculated with a seeding density of 3 x 10^5/mL	1.0 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 0.5 % and 1.5 % should be tested as well	0.1 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 0.05 % and 0.15 % should be tested as well
Cell line B is growing to high peak cell densities of 5 x 10^7 cells/mL, reached on day 10 and was inoculated with a seeding density of 1.2 x 10^6/mL	2.5 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 2.0 % and 3.0 % should be tested as well	0.25 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 0.20 % and 0.30 % should be tested as well
Cell line C is growing to high intermediate cell densities of 2 x 10^7 cells/mL, reached on day 12 and was inoculated with a seeding density of 1.2 x 10^6/mL	2.0 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 1.5 % and 2.5 % should be tested as well	0.20 % (v/v of initial volume per day) starting on day 3 of cultivation, feed rates of 0.15 % and 0.25 % should be tested as well

Note: A fixed feed rate is recommended, however, a dynamic feed rate can also be beneficial, especially for cell lines that show a long lag-phase and start to grow exponentially in a later phase of the fed-batch. The feed rate can be increased accordingly in this later phase.

Application of First CHOice® LG Medium w.o. HT/ First CHOice® LG Medium Plus w.o. HT

First CHOice® LG Medium w.o. HT and First CHOice® LG Medium Plus w.o. HT were developed to reduce galactosylated N-glycan species in manufacturing processes. A matching glycan profile is of strong interest in the context of biosimilar development. Both products can be used individually or be blended with First CHOice® Medium w.o. HT or First CHOice® Medium Plus 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: First CHOice® LG products need to be supplemented accordingly (please refer to "Preparation of complete growth medium") with HT supplement, L-glutamine and anti-clumping agent on demand. A prior adaptation to these media may be beneficial. Please refer to "Adaptation of CHO cells to First CHOice® media", Option 2 for more details. First CHOice® LG products do not affect cellular growth, productivity or other product quality parameters significantly.



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