

CHOventure

MEDIA SYSTEM:

INSTRUCTIONS FOR USE



CHOventure Instructions for Use

Adaptation and Expression Instructions for CHOventure Media System

Introduction

CHOventure is a chemically defined media system developed in cooperation with ExcellGene SA. It is specifically tailored to CHO cell lines for maximum quality and yield of recombinant protein production. The system consists of a growth medium complemented by two feeding supplements CHOventure Feed A & Feed B. It is tailored to the usage of high-density cell cultures promoting uniform cell distribution as well as preventing cell lethality. CHOventure features robust and scalable growth performance, from small-scale experiments to large-scale bioreactors in batch- and fed-batch systems.

CHOventure in Batch- and Fed-Batch Systems

To efficiently optimize expression titers with CHOventure, production in fed-batch reactor systems is recommended supplementing the growth medium with the CHOventure Feed A & Feed B. The Two-feed system allows for combinatorial control of custom-tailored nutrient supply for every CHO cell line.

CHOventure Feed A contains an enriched mixture of essential amino acids, vitamins, and co-factors to create an optimal environment for robust cell growth. CHOventure Feed B is a concentrated amino acid composition designed to complement the expression system.

These instructions for use provide detailed information about adaptation to CHOventure medium as well as feeding strategies for successfully achieving maximum expression titers with this cutting-edge media system.

CHOventure General Conditions

Application	Efficient production of recombinant proteins, biosimilars, and other pharmaceutical products
Suitable Cell Lines	Most CHO cell lines and derivatives, such as CHO DG44, CHO-K1, CHO-S, CHO-DXB11, ExpiCHO, CHOExpress®, and many more
Reactor Types	Batch & fed-batch systems in analytical- to large-scale bioreactors

Components of the CHOventure Media System

CHOventure Media System consists of a growth medium and two feeds. All components are available in liquid and powder format.

For effectively using the CHOventure system, it is essential to supplement the medium with L-Glutamine. CHOventure growth medium contains 7 g/L glucose for optimal mitotic progression of CHO cells. It is recommended to supplement cultures with additional glucose when concentration is below 2 g/L. Insulin may be supplemented to the cultures at concentrations of 5 mg/L. Other supplements may be added on process and cell line specific considerations.

CHOventure Liquid Products

CHOventure Growth Medium, with Hypoxanthine, with Thymidine, with Pluronic™, w/o Insulin, w/o L-Glutamine	500 ml Bag 10 L Bag 20 L	VEN-500ML On request On request
CHOventure Feed A, Feeding Supplement for CHO Cells, w/o Insulin, w/o L-Glutamine	500 ml 100 ml	VENFA-500ML VENFA-100ML
CHOventure Feed B, Feeding Supplement for CHO Cells, w/o Insulin, w/o L-Glutamine	50 ml 10 ml	VENFB-50ML VENFB-10ML

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CHOventure Starter Kit Including 1 x CHOventure Growth Medium (500 ml) 2 x CHOventure Feed A (100 ml) 2 x CHOventure Feed B (10 ml)	Kit	VEN-K1
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CHOventure Powder Products

CHOventure Growth Medium, Powder, with Hypoxanthine, with Thymidine, with Pluronic™, w/o Insulin, w/o L-Glutamine	10 L 1 L	VEN-P10 VEN-P1
CHOventure Feed A, Powder, Feeding Supplement for CHO Cells, w/o Insulin, w/o L-Glutamine	10 L 1 L	VENFA-P10 VENFA-P1
CHOventure Feed B, Powder, Feeding Supplement for CHO Cells, w/o Insulin, w/o L-Glutamine	1 L	VENFB-P1

Supplements

Glucose Solution (250 g/L)	50 ml	GLC-F
L-Glutamine (200 mM)	100 ml	GLN-B
Recombinant Insulin (5 mg/ml)	5 ml	INS-K

Adaptation to CHOventure Growth Medium

For robust CHO cells grown in a different medium, no adaptation is needed, and cells may be directly transferred into CHOventure growth medium. It is advisable to keep a backup culture in the original medium until cells have adapted. For sensitive CHO cells, it is possible to observe suboptimal growth after direct adaptation for 3 – 5 passages. In this case sequential adaptation method is recommended. Additional supplements may be added on process and cell line specific considerations.

Sequential Adaptation

Culture Flask Type	125 ml shake flask
Medium Volume	25 ml
Inoculation Cell Density	5×10^5 cells/ml
Shaking Rate	100 – 150 rpm
Temperature	37°C
CO ₂ Concentration	5.0%

- Subculture cells into 6.25 ml of supplemented CHOventure medium mixed with 18.75 ml of the original medium (1:4 ratio). During the adaptation procedure, seed at twice the normal seeding density. Subculture cells when confluency reaches 70 – 90%.

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- Once consistent cell growth with high viability has been achieved, passage cells into fresh medium with an increased concentration of CHOventure. Perform adaptation using the following mixture compositions:

Step	Ratio	Volume CHOventure (ml)	Volume Original Medium (ml)
1	1:4	6.25	18.75
2	1:2	12.5	12.5
3	3:4	18.75	6.25
4	9:10	22.5	2.5
5	1:1	25	0

Multiple passages at each step of adaptation may be needed.

- Continue to monitor and passage cells until consistent growth with high viability is achieved. After several passages in 100% new medium, the culture is adapted.

Cell adaptation is strongly recommended before performing expression experiments in batch or fed-batch reactors.

CHOventure Fed-Batch Performance Test

Protein expression in general harbours a variety of parameters for optimization. Identifying the optimal feeding strategy will enable achieving the highest yields for the used cell line and system. When performing test expression experiments using CHOventure we recommend the following Feeding Strategy setup as a starting point for further adaptations:

Fed Batch

Culture Flask Type	125 ml shake flask
Starting Volume	25 ml
Basal Medium	CHOventure Growth Medium
Feed Supplements & Strategy	CHOventure Feed A: Feed 4.0% of total volume each day, starting at day 3 of fed-batch experiment
	CHOventure Feed B: Feed 0.4% of total volume each day, starting at day 3 of fed-batch experiment
	Feeding Option 1: for initial testing, feed culture with 4.0% Feed A and 0.4% Feed B
Alternative Feeding Strategies	If glucose accumulates in the culture, try Feeding Option 2 and Option 3:
	Feeding Option 2: for slow growing cells, feed culture with 3.0% Feed A and 0.3% Feed
	Feeding Option 3: for very slow growing cells, feed culture with 2.0% Feed A and 0.2% Feed B
Supplements	Glucose Recommended Culture Concentration: 1 – 5 g/L When concentration falls below 2 g/L, add glucose to a final concentration of 5 g/L)

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Supplements	L-Glutamine Recommended Concentration: 4 mM Supplementing growth medium with L-Glutamine is essential for CHO cell viability
	Insulin Recommended Concentration: 5 mg/L in CHOventure Growth Medium The need for insulin is cell line dependent
Inoculation Cell Density	5 x 10 ⁵ cells/ml
Culture Duration	14 days, for optimal results 17 days
Harvest Criteria	Cell viability < 60%
Shaking Rate	100 – 150 rpm
Temperature	37°C [day 0 – day 4]; 34°C [from day 4]
CO ₂ Concentration	5.0%

- **Feeding Option 1:** Starting on the third day; 4.0% of CHOventure Feed A and 0.4% of CHOventure Feed B of the total volume of the culture is added to the cells.
- For CHO cells exhibiting slower growth, reduced feeding strategy (option 2 and option 3) is recommended. Reduced feeding in slow growing cell cultures can promote accurate protein folding, avoiding aggregation and insolubility while preventing over-feeding of cells.
Feeding Option 2: Starting on the third day; 3.0% of CHOventure Feed A and 0.3% of CHOventure Feed B of the total volume of the culture is added to the cells.
Feeding Option 3: Starting on the third day; 2.0% of CHOventure Feed A and 0.2% of CHOventure Feed B of the total volume of the culture is added to the cells.
- Shake the culture during feeding with the feed supplements to ensure uniform distribution.

Depending on cell growth and production yield, reduce or increase the feed amount in the next experiment.

General Notes

- It is not necessary to count the CHO cells the first two days. The first time point of cell count is day 3 after inoculation.
- Sample your culture each day before feeding. It is highly recommended to monitor the following parameters: Total cell concentration, viable cell density (VCD), pH, as well as concentration of glucose, lactate, and produced protein of interest.
- If you use a Cedex system for cell counting, it is possible that the cell count will be disturbed by colloid formation. This problem can be overcome by 1:3 diluting the sample in PBS (e.g., 0.33 ml CHO cell solution, 0.67 ml PBS) and counting thereafter.
- Harvest cells if **cell viability** is below 60%.
- Due to the nutrient-rich formulation of this product, the formation of visible precipitates is possible. However, these have not shown any negative influence on the performance of the media system.

Technical Support

Protein expression may be challenging, and various optimizations can be taken into account for improved titer expression. Besides optimal medium composition and feeding strategy, also physical parameters such as temperature may affect cell viability and proper protein folding. For technical support, feel free to contact our experts at techservice@capricorn-scientific.com or phone (+49 6424 944640).



WHY US?

» Your Partner in Cell Culture

We are a company dedicated to cell culture. Our specialists offer their experience to support your manufacturing processes.

» Competence, Commitment, and Improvement

Our ISO 9001:2015 certification confirms that we are regularly audited and certified by an independent organization, to continuously improve our quality system and our standards, processes, and products.

» Fast and Efficient Order Processing

Secured just-in-time delivery due to short administrative channels and good knowledge on product quality, stability, and delivery conditions.

» Customized Production and Development

We design your media according to your recipe, or create innovative solutions that can improve the performance and efficiency of your specific processes.

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