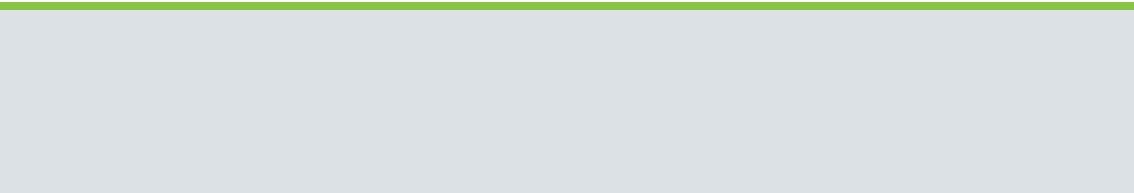




CHO|ONE FlexKit

FEED INSTRUCTION FOR USE



CHO|ONE Feed FlexKit Instructions for Use

Instruction for Cultivation & Fed-Batch Expression

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Introduction

CHO cell lines exhibit distinct metabolic profiles and nutrient requirements depending on clone, expression system, and process conditions. Consequently, a single standard medium rarely delivers optimal performance across different CHO systems.

The CHO|ONE platform addresses this challenge through a structured workflow consisting of the CHO|ONE Media FlexKit for identification of an optimal expression medium and the CHO|ONE Feed FlexKit for subsequent evaluation of efficient feeding strategies in fed-batch processes. Following Step 1, the selection of the CHO|ONE Expression Medium B, L, or X, the CHO|ONE Feed FlexKit represents Step 2 of this workflow and enables systematic assessment of feed formulations in combination with the selected medium.

From Screening to Precision Media – Proceeding with Step 2 of Your Workflow

CHO EXPERT CONSULTING SERVICE

STEP 1

Media Screening with the CHO|ONE Media FlexKit

Evaluate all CHO|ONE media formulations in parallel to assess baseline performance of your CHO clone and identify the best-suited expression medium.

STEP 2

Feed Strategy Screening with the CHO|ONE Feed FlexKit

Screen different feeds of the CHO|ONE Feed FlexKit with your selected CHO|ONE expression medium to define your tailored fed-batch strategy.

STEP 3

Custom Media and Feed Development

Based on previous result, further customized CHO|ONE formulations can be developed to precisely match your CHO clone's metabolic and production requirements.



At any stage during media screening or feed strategy development you may engage our support to optimize performance, robustness, and reproducibility.

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In fed-batch cultivation, implementation of an appropriate feeding strategy is an essential part of process design, directly impacting cell growth, metabolic performance, and product yield. The CHO|ONE Feed FlexKit provides multiple feed formulations to address different metabolic demands of CHO cell lines:

Variants of Feed 1	Variants of Feed 2
<p>CHO ONE Feed 1A — <i>Basal formulation with a balanced nutrient concentration</i></p> <p>CHO ONE Feed 1B — <i>Modified salts balancing osmolality and metabolite accumulation</i></p> <p>CHO ONE Feed 1C — <i>Reduced nutrient concentrations for sensitive CHO clones</i></p>	<p>CHO ONE Feed 2 — <i>One standardized version boosting metabolic support</i></p>

The CHO|ONE Feed FlexKit supports identification and evaluation of process-specific feeding strategies for fed-batch production. By testing the CHO|ONE Feed 1 variants combined with CHO|ONE Feed 2, users can establish a feeding strategy tailored to the requirements of their clone in fed-batch applications.

These instructions for use provide guidance for the application of the CHO|ONE Feed FlexKit and for the evaluation of feeding strategies in fed-batch processes.

CHO|ONE Application Overview

Application	Adaptation, selection, and growth of CHO Cells for further production processes of recombinant proteins, biosimilars, and other pharmaceutical products
Suitable Cell Lines	Optimized for CHO DG44 but also for other cell lines like CHO-K1, CHO-S
Reactor Types	Batch & fed-batch systems from small scale to large-scale bioreactors

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Components of the CHO|ONE Feed FlexKit

The CHO|ONE Feed FlexKit consists of three variants of CHO|ONE Feed 1 and one variant of CHO|ONE Feed 2.

For fed-batch applications, one of the variants of CHO|ONE Feed 1 is used in combination with CHO|ONE Feed 2, together with the selected CHO|ONE expression medium (CHO|ONE B, L, or X). During cultivation, the expression medium is additionally supplemented as required to maintain optimal nutrient levels.

Product Name	Volume	Cat. No.
CHO ONE Feed 1A Feeding Supplement for CHO Cells with Pluronic™, w/o Insulin, w/o L-Glutamine	250 ml	CHOF1A-250ML
CHO ONE Feed 1B Feeding Supplement for CHO Cells with Pluronic™, w/o Insulin, w/o L-Glutamine	250 ml	CHOF1B-250ML
CHO ONE Feed 1C Feeding Supplement for CHO Cells with Pluronic™, w/o Insulin, w/o L-Glutamine	250 ml	CHOF1C-250ML
CHO ONE Feed 2 Feeding Supplement for CHO Cells w/o Insulin, w/o L-Glutamine	75 ml	CHOF2-75ML
CHO ONE Feed FlexKit Composition of different variants of CHO ONE Feeding Supplements	Kit	CHOF-K1

*Products are also available in powder form.

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Optional Supplements (not included)

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

CHO|ONE Fed-Batch Performance Test

Protein expression in general provides 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 CHO|ONE we recommend the following Feeding Strategy setup as a starting point for further adaptations:

General Culture Parameter

Culture Flask Type	125 ml shake flask
Starting Volume	25 ml
Your Basal Medium	According to Step 1 of your workflow: CHO ONE B or CHO ONE L or CHO ONE X
Inoculation Cell Density	3×10^5 cells/ml
Culture Duration	Max. 17 days
Harvest Criteria	Cell viability < 60%
Shaking Rate	Orbital Shaking: 100 – 150 rpm Linear Shaking: 110 rpm
Temperature	37.0°C
CO ₂ Concentration	5.0%

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General Strategy for Fed-Batch Cultivation

Feeding Schedule	<ul style="list-style-type: none">• Do not feed at day 1 and 2• Feed each day, starting on day 3• Take samples corresponding to the volume of feeds to be added each day starting on day 3• Feed after daily sampling• Add Feed 1 & Feed 2 at different times points
Feed Supplements & Strategy	<p>CHO ONE Feed 1 A/B/C: Feed 3.0% of total volume each day, starting on day 3</p> <p>CHO ONE Feed 2: Feed 0.3% of total volume each day, starting on day 3</p>
Supplements	<p>Glucose Recommended Concentration: 4.0 g/L When concentration falls below 2.0 g/L, add glucose to a final concentration of 4.0 g/L)</p>
	<p>L-Glutamine Recommended Concentration: 6 mM Supplementing CHO ONE Medium with L-Glutamine is essential for CHO cell viability</p>
	<p>Insulin Recommended Concentration: 5 mg/L in CHO ONE Medium. The need for insulin is cell line dependent</p>

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General Notes:

- It is not necessary to count the CHO cells the first two days. The first time point of cell count is on 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 the concentration of glucose is too low, additional glucose should be added. If the concentration is too high, the percentage of feed should be reduced or feeding should be skipped for one day.
- 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.

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Initial Feeding Strategy: Test Conditions to Start With

The CHO|ONE Feed FlexKit enables a wide range of possible feeding strategies due to the combinatorial use of different feed formulations and process conditions. To ensure a structured and efficient starting point, it is recommended to initially evaluate the different feed formulations under standardized conditions.

Each CHO|ONE Feed 1 variant should be tested in combination with CHO|ONE Feed 2 using the selected CHO|ONE expression medium (CHO|ONE B, L, or X). All feeds should be applied at the recommended standard concentrations:

	Total Culture Volume	Feed Volume to be added each Day			
Approach	Medium [ml]	Feed 1A [ml]	Feed 1B [ml]	Feed 1C [ml]	Feed 2 [ml]
A	25	0.75	-	-	0.075
B	25	-	0.75	-	0.075
C	25	-	-	0.75	0.075

Example for an initial feeding schedule:

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	...
Feed 1 [%]				3	3	3	3	3	3	3	3	3	3	3	3	3
Feed 2 [%]				0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

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Based on the obtained results of the initial feeding testing, process-specific adjustments may be performed to further optimize cell growth, metabolic performance, and product yield.

CHO|ONE Feeding Options:

<p>Feeding Strategy</p>	<p>To improve growth and productivity, fine-tuning feeding strategy is recommended. Reduced feeding in slow growing cell cultures can promote accurate protein folding, while fast growing cells require increased nutrients</p> <p>CHO ONE Feed 1 A/B/C: Feed 1.0 – 4.0% of total volume each day, starting on day 3</p> <p>CHO ONE Feed 2: Feed 0.1 – 0.4% of total volume each day, starting on day 3</p>
<p>Feed Composition</p>	<p>CHO ONE Feed 1 A, Feed 1B and Feed 1C can be mixed in different ratios to obtain newly formulated, tailored feeding supplements, initially starting with a 50%/ 50% mixture of Feed 1A & Feed 1B or Feed 1A & Feed 1C or Feed 1B & Feed 1C.</p> <p>Optimized ratios can subsequently be refined based on process performance, for example by testing adjusted proportions such as 60% / 40% mixtures.</p>

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CHO|ONE Process Optimization

Fed-batch performance is closely linked to nutrient supply and metabolic balance. Deviations in growth, viability, productivity, or metabolite levels often indicate suboptimal feeding conditions.

The following table supports identification of process limitations and provides guidance for targeted adjustments, including selection and optimization of feeding strategies using the CHO|ONE Feed FlexKit.

Process Optimization Guide:

If you observe	Check for	Interpretation	Action
High lactate	Residual glucose high	Carbon overload	Reduce glucose setpoint and/or feed intensity; consider switching to Feed 1C
	Osmolality increasing	Metabolic overload	Reduce feed intensity; consider Feed 1B to improve osmotic balance
Low VCD	Lactate high	Inefficient metabolism	Reduce glucose input; evaluate Feed 1C for sensitive clones
	Lactate low + glucose low	Nutrient limitation	Increase feeding or adjust schedule; consider Feed 1A
	Ammonium high	Nitrogen overload	Reduce glutamine input; consider reducing overall feed load (prefer Feed 1C)
Low viability	Lactate high	Metabolic stress	Reduce feed intensity; switch to Feed 1C
	Osmolality high	Osmotic stress	Reduce feed and salt load; evaluate Feed 1B

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If you observe	Check for	Interpretation	Action
Low productivity	Low VCD	Insufficient biomass	Optimize early feeding; consider Feed 1A to support growth
	High VCD + good viability	Production limitation	Maintain feeding; evaluate process parameters (e.g. temperature shift)
	High ammonium	Metabolic inhibition	Reduce glutamine and overall nutrient load; consider Feed 1C
Cell aggregation	Occurs after feeding	Local overfeeding	Reduce volume per feeding event; improve mixing; consider Feed 1C
Protein aggregation	High osmolality	Osmotic stress	Reduce feed load; consider Feed 1B to control osmolality

For support in experimental design or data interpretation, Capricorn Scientific’s technical experts are available at any stage of the process.

Precautions and Disclaimer

This product is for research use and further manufacturing only.
 Pluronic is a trademark of BASF Corporation.

Technical Support

At any stage during media and feed screening, you may engage our support to optimize performance, robustness, and reproducibility. 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.

Capricorn Scientific GmbH
Auf der Lette 13 A
35085 Ebsdorfergrund
Germany

Tel.: +49 6424 944 64 0
Fax: +49 6424 944 64-20

info@capricorn-scientific.com
techservice@capricorn-scientific.com

www.capricorn-scientific.com

