



# CHO|ONE FlexKit MEDIA

INSTRUCTION FOR USE



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# CHO|ONE Media FlexKit Instructions for Use

## Instruction for Adaptation and Cultivation

### Overview

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Introduction .....	3
Components of the CHO ONE Media FlexKit .....	4
Adaptation to CHO ONE Media FlexKit .....	5
General Notes .....	7
Precautions and Disclaimer .....	8
Technical Support .....	8
Notes .....	9

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## Instruction for Adaptation and Cultivation

### Introduction

CHO cell lines exhibit distinct metabolic profiles and nutrient demands depending on clone, expression system, and process conditions. A single standard medium rarely delivers optimal performance across all systems.

The CHO|ONE Media FlexKit addresses this challenge by offering three different media formulations in one Testkit:

- CHO|ONE B — *Basal formulation with a balanced nutrient concentration*
- CHO|ONE L — *Light formulation with reduced amino acid and glucose levels*
- CHO|ONE X — *High concentration level of amino acids and glucose*

By testing these media variants in parallel, users can efficiently identify the formulation that supports their specific CHO cell line and process requirements.

### 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 in analytical- to large-scale bioreactors

## CHO|ONE Media FlexKit Instructions for Use

### Instruction for Adaptation and Cultivation

#### Components of the CHO|ONE Media FlexKit

The CHO|ONE Media FlexKit consists of three variants of chemically defined expression media. For effectively using the CHO|ONE Media, it is essential to supplement the medium with L-Glutamine (6 mM recommended). CHO|ONE expression media contain glucose for optimal mitotic progression of CHO cells. When measured glucose level is below 2 g/L, it is recommended to supplement cultures with additional glucose to a final concentration of 4 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.

Product Name	Volume	Cat. No.
CHO ONE B, Expression Medium, with Pluronic™, w/o HT, w/o Insulin, w/o L-Glutamine	500 ml	CHOB-500ML
CHO ONE L, Expression Medium, with Pluronic™, w/o HT, w/o Insulin, w/o L-Glutamine	500 ml	CHOL-500ML
CHO ONE X, Expression Medium, with Pluronic™, w/o HT, w/o Insulin, w/o L-Glutamine	500 ml	CHOX-500ML
CHO ONE Media FlexKit: 1 x CHO ONE B (500 ml) 1 x CHO ONE L (500 ml) 1 x CHO ONE X (500 ml)	Kit	CHOM-K1

\*Products are also available in powder form.

#### 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 Media FlexKit Instructions for Use

## Instruction for Adaptation and Cultivation

### Adaptation to CHO|ONE Media FlexKit

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We recommend to evaluate all CHO|ONE media formulations in parallel under identical conditions to assess adaptation and baseline performance of your CHO cell clone and identify the best-suited expression medium.

For robust CHO cells grown in a different medium, no adaptation is needed, and cells may be directly transferred into CHO|ONE expression media. It is advisable to keep a backup culture in the original medium until cells have adapted.

Culture Flask Type	125 ml shake flask
Medium Volume	25 ml
Inoculation Cell Density	$3 \times 10^5$ cells/ml
Shaking Rate	100 – 150 rpm (orbital) 110 rpm (linear)
Temperature	37°C
CO <sub>2</sub> Concentration	5.0%
Culture Duration	3 days
Target viable cell concentration at the end of culture	$> 1 \times 10^6$ cells/ml*

\* If viable cell concentration is  $< 6 \times 10^5$  cells/ml, centrifuge the cells and re-suspend the pellet in fresh medium (centrifugation conditions: 190 x g, 3 min, room temperature), otherwise do not centrifuge.

Additional supplements may be added on process and cell line specific considerations.

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.

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## CHO|ONE Media FlexKit Instructions for Use

### Instruction for Adaptation and Cultivation

#### Sequential Adaptation

- Subculture cells into 6.25 ml of supplemented CHO|ONE 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 confluence reaches 70 –90%.
- Once consistent cell growth with high viability has been achieved, passage cells into fresh medium with an increased concentration of CHO|ONE. Perform adaptation using the following mixture compositions:

Step	Ratio	Volume CHO ONE (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	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.

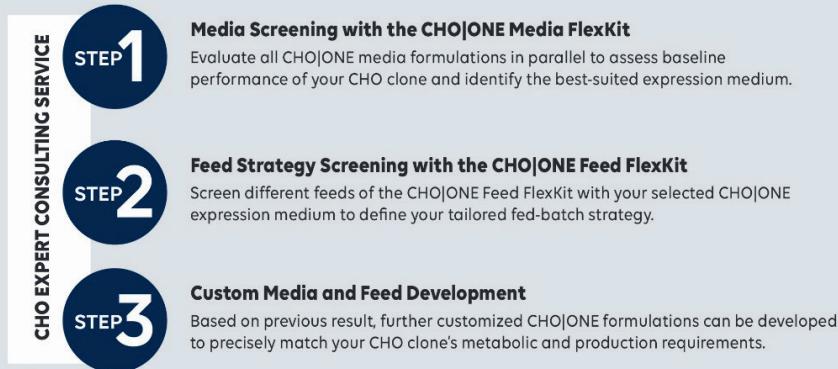
# CHO|ONE Media FlexKit Instructions for Use

## Instruction for Adaptation and Cultivation

### 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.
- Important parameters to measure at sampling days: Total cell concentration, viable cell density (VCD), pH, 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.

### From Screening to Precision Media — All in One Workflow



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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## CHO|ONE Media FlexKit Instructions for Use

### Instruction for Adaptation and Cultivation

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#### Precautions and Disclaimer

This product is for research use and further manufacturing only.

Pluronic is a trademark of BASF Corporation.

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#### Technical Support

At any stage during media 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](mailto:techservice@capricorn-scientific.com) or phone (+49 6424 944640).

## CHO|ONE Media FlexKit Instructions for Use

## Instruction for Adaptation and Cultivation

## Notes





## 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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