CAPRICORN SCIENTIFIC

Process for Success

# CHO|ONE Media System™

## Instruction for Use

| Product Name | Volume | Cat. No. |
|---|---|---|
| CHO\|ONE Media System™, Starter Kit | | CHO-K1 |
| CHO\|ONE Media System™, Expansion Kit | | CHO-K2 |
| CHO\|ONE A | 500 ml | CHOA-500ML |
| CHO\|ONE E | 500 ml / 1000 ml | CHOE-500ML / CHOE-1000ML |
| CHO\|ONE Feed 1 | 100 ml / 500 ml | CHOF1-100ML / CHOF1-500ML |
| CHO\|ONE Feed 2 | 10 ml / 50 ml | CHOF2-10ML / CHOF2-50ML |

## Test Kit Composition

| Liquid Kit Component | Starter Kit | Expansion Kit |
|---|---|---|
| CHO\|ONE A | 1 x 500 ml | |
| CHO\|ONE E | 1 x 500 ml | 5 x 1000 ml |
| CHO\|ONE Feed 1 | 2 x 100 ml | 5 x 500 ml |
| CHO\|ONE Feed 2 | 2 x 10 ml | 5 x 50 ml |

### Composition

CHO|ONE Media System™ is chemically defined, free of hydrolysates, animal component free (ACF) and contains Pluronic™. This system does not contain L-glutamine and phenol red.

### Shelf Life and Storage

Shelf Life: The shelf life of the kit is 18 months after manufacturing.

For individual shelf life please refer to the product label.

Storage: +2 °C to +8 °C.

### Applications

- For CHO-S, CHO-DG44 & CHO-K1 cells
- Fed-batch culture of CHO cells
- Selection, adaptation and production

### CHO|ONE Media™

CHO|ONE A allows the cultivation or adaptation of recombinant CHO cells in small-scale shaker bottles or spinner flasks. In addition, its formulation simplifies and shortens the time-consuming adaptation to fed-batch culture.

CHO|ONE E allows the cultivation of recombinant CHO cells for fed-batch cultures in spinner flasks or large-scale bioreactors. It can be used for effective recombinant CHO cells achieving high production yields of active protein.

### Fed-batch with the CHO|ONE Media System™

For optimal results the CHO|ONE E medium has to be used in combination with the feeding additives CHO|ONE Feed 1 and CHO|ONE Feed 2. The use of CHO|ONE E and CHO|ONE feeding supplements allows mixing or combinatorial approaches.

For fed-batch culture optimization CHO|ONE E must be supplemented with CHO|ONE Feed 1 (starting with 1/25 volume of CHO|ONE E) and CHO|ONE Feed 2 (starting with 1/10 volume of CHO|ONE Feed 1) daily (or at other time intervals). For further procedure see fed-batch performance and feeding strategies below.

For large scale fed-batch please scale up and use the ratio from small scale optimization.

For in vitro laboratory use or further manufacturing only.
Pluronic™ is a registrated trademark of BASF.

**Test options**

The media are designed for fed-batch cultures. There are several options to test the media:

1. Fed-batch with previous cell adaptation

If cell adaptation is preferred before fed-batch culture, take an aliquot from your stock culture. Inoculate the cells in CHO|ONE A by simply splitting them into the new medium. Split the cells according to the description below for several passages. Use these cells as inoculum for fed-batch performance.

2. Fed-batch without cell adaptation

Some cells grow in CHO|ONE E without previous adaptation. As a prerequisite, your cells shall grow regularly to the expected viable cell concentration and viability shall remain high over several passages in your medium. Take an aliquot from your stock culture and inoculate directly in the new production medium CHO|ONE E.

3. Fed-batch to test only the feed supplements

If using a different production medium, test only the optimized feed supplements CHO|ONE Feed 1 and CHO|ONE Feed 2 for fed-batch culture.

This CHO|ONE Media System™ was developed to enhance the performance of CHO cells for protein production. The different components were developed to complement each other. Therefore, in order to benefit from the whole system, we recommend Option 1 or 2.

**General culturing**

Take an aliquot of cells growing in your culture medium. Inoculate a shake flask by splitting the cells into the adaptation medium. Do not centrifuge the cells for inoculation.

Sub-culture the cells every 3 days. If the cells start growing to a final cell concentration of >12 x $10^5$/ml in the end of 3-days-culture, the cell adaptation is completed and you can start with the fed-batch process.

## Cell adaptation

| | |
|---|---|
| Culture flask type | Shake flask |
| Total volume | 125 ml |
| Working volume | 25 ml |
| Shaking rate | 100-150 (orbital) rpm<br>110 (linear) rpm |
| Inoculation cell density | 3 x $10^5$ cells/ml |
| Culture duration | 3 days |
| Target viable cell concentration at the end of culture | > 1 x $10^6$ cells/ml* |
| Temperature | 36.8 ± 0.2°C |
| $pCO_2$ | 7.5% |

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

## Fed-batch performance

| | |
|---|---|
| Culture flask type | Shake flask |
| Total volume | 125 ml |
| Working volume | 25 ml |
| Shaking rate | 100-150 (orbital) rpm<br>110 (linear) rpm |
| Basal medium | CHO\|ONE E; add L-Glutamine (recommended 6 mM) |
| Feed supplements | CHO\|ONE Feed 1 and CHO\|ONE Feed 2 |
| Inoculation cell density | 3 x $10^5$ cells/ml |
| Culture duration | max. 17 days |
| Harvest criteria | Viability <60% |
| Temperature | 36.8 ± 0.2°C |
| $pCO_2$ | 7.5% |
| Sampling | Days of feeding only; take 1 ml or less |
| Sampling days | 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| Feeding time point | After taking sample for cell count |
| Feeding days | 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 |
| Feed amount | Test two different feeding modi as described in the table below |
| Glucose addition | When daily measured glucose of culture is <2 g/l, bring glucose to a final concentration of 4 g/l. |
| pH value | Keep at pH 7.1 |

- Do not count cells the first 3 days. The first time point of cell count is day 3 after inoculation.
- Important parameters to measure at sampling days: Viable cell concentration (viability), total cell concentration, glucose, lactate, pH, protein to be expressed.
- If the cells grow well in fed-batch (peak cell concentration > 1 x $10^7$/ml), the first feeding option is applied (0.5 ml/flask CHO|ONE Feed 1 and 0.05 ml/flask CHO|ONE Feed 2). The weaker the cells grow, the less feed is required. Therefore, the second feeding option can be applied (for example, 0.75 ml/flask CHO|ONE Feed 1 and 0.075 ml/flask CHO|ONE Feed 2). Start testing the two feeding patterns shown in the following tables. Depending on cell growth, reduce or increase the feed amount in the next experiment.
- Shake the culture during feeding with the feed supplements.
- 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 diluting the sample in PBS (one part cell-solution, two parts PBS) and counting thereafter.

## Recommended feeding strategies

**First feeding option (normal growing cells)** (peak cell concentration > 1 x $10^7$ /ml):

25 ml CHO|ONE E

+ 0.5 ml CHO|ONE Feed 1

+ 0.05 ml CHO|ONE Feed 2

| Culture day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHO\|ONE Feed 1 (ml/flask) | - | - | - | 0,5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| CHO\|ONE Feed 2 (ml/flask) | - | - | - | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

**Second feeding option (fast growing cells):**

25 ml CHO|ONE E

+ 0.75 ml CHO|ONE Feed 1

+ 0.075 ml CHO|ONE Feed 2

| Culture day | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHO\|ONE Feed 1 (ml/flask) | - | - | - | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| CHO\|ONE Feed 2 (ml/flask) | - | - | - | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 | 0.075 |

**For further feeding options please use other ratios:**

- **Third option (very slow growing cells):**
  25 ml CHO|ONE E
  + 0.425 ml CHO|ONE Feed 1
  + 0.0425 ml CHO|ONE Feed 2

- **Fourth option (very fast growing cells):**
  25 ml CHO|ONE E
  + 1.0 ml CHO|ONE Feed 1
  + 0.1 ml CHO|ONE Feed 2

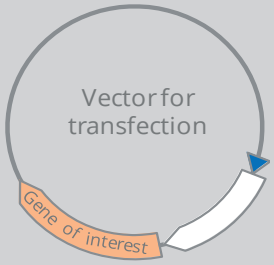
For large scale fed-batch production please use the ratio of small scale optimization.

## Additives

| Product | Cat. No. | Volume | Recommended concentration | Add to |
|---|---|---|---|---|
| L-Glutamine, (200 mM) | GLN-B | 100 ml | 6 mM | CHO\|ONE E & A |
| Recombinant Insulin (5 mg/ml) | INS-K | 5 ml | 5 mg/l (A & E)<br>10 mg/l (Feed 1) | CHO\|ONE E & A & Feed 1 |
| Glucose, (250 g/l) | GLC-F | 50 ml | > 2 g/l | Fed-batch culture |

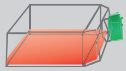
## Flow chart CHO|ONE Media System™

1. Transfection



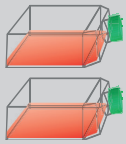

Transformation in CHO cells

2. Selection



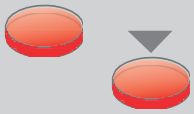
First selection

3. Recovery and Expansion



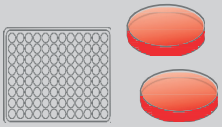
Recovery and expansion

4. Amplification



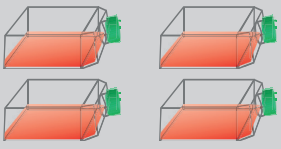
Gene amplification with substances

5. Screening



Second selection

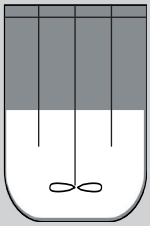
6. Expansion



Recovery and expansion in CHO|ONE A medium

Final adaptation in CHO|ONE A medium for fed-batch optimisation

7. Growth evaluation & process optimization



**Fed-batch optimisation strategy**
Fed-batch optimization in CHO|ONE E medium supplemented with CHO|ONE Feed 1 and CHO|ONE Feed 2

Large scale adaptation with CHO|ONE E medium supplemented with CHO|ONE Feed 1 and CHO|ONE Feed 2