CHO CD EfficientFeed™ Kit
Chemically Defined Feed System for CHO Cell Cultures
Catalog nos. A10241-01, A10234-01, A10240-01
Version A
2 October 2007
Part no. A10276
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Kit Contents and Storage

Shipping/Storage

The components of the CHO CD EfficientFeed™ Kit are shipped and stored as described in the table below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Cat. No.</th>
<th>Shipping</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO CD EfficientFeed™ Kit</td>
<td>A10241-01</td>
<td>Ambient</td>
<td>See below</td>
</tr>
<tr>
<td>CHO CD EfficientFeed™ A</td>
<td>A10234-01</td>
<td>Ambient</td>
<td>2-8°C, protected from light</td>
</tr>
<tr>
<td>CHO CD EfficientFeed™ B</td>
<td>A10240-01</td>
<td>Ambient</td>
<td>2-8°C, protected from light</td>
</tr>
</tbody>
</table>

CHO CD EfficientFeed™ A

Amount supplied: 1 liter
Composition: Concentrated, chemically defined, membrane-filtered liquid formulation reconstituted from AGT™, plus additional supplements.

Contains:
Carbon source
Concentrated amino acids
Vitamins
Salts
Trace minerals

Does not contain:
Lipids, hydrolysates, or growth factors
Protein free

CHO CD EfficientFeed™ B

Amount supplied: 1 liter
Composition: Concentrated, chemically defined, membrane-filtered liquid formulation reconstituted from AGT™, plus additional supplements.

Contains:
Carbon source
Concentrated amino acids
Vitamins
Salts
Trace minerals

Does not contain:
Lipids, hydrolysates or growth factors
Protein free
Accessory Products

Introduction

The products listed in this section may be used with the CHO CD EfficientFeed™ Kit. For more information, refer to our Web site (www.invitrogen.com/bioproduction) or call Technical Service (see page 13).

Accessory Products

The following reagents supplied in the CHO CD EfficientFeed™ Kit and other reagents suitable for use with the kit are available separately from Invitrogen. Ordering information is provided below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO CD EfficientFeed™ A</td>
<td>1,000 mL</td>
<td>A10234-01</td>
</tr>
<tr>
<td>CHO CD EfficientFeed™ B</td>
<td>1,000 mL</td>
<td>A10240-01</td>
</tr>
<tr>
<td>CD OptiCHO™ Medium</td>
<td>1,000 mL</td>
<td>12681-011</td>
</tr>
<tr>
<td>CD CHO Medium</td>
<td>1,000 mL</td>
<td>10743-029</td>
</tr>
<tr>
<td>GlutaMAX™</td>
<td>100 mL</td>
<td>35050-061</td>
</tr>
<tr>
<td>FoamAway™ Irradiated</td>
<td>0.5 L in 1 L universal bag</td>
<td>0060096BC</td>
</tr>
<tr>
<td></td>
<td>2.5 L in 5 L universal bag</td>
<td>0060096BA</td>
</tr>
<tr>
<td>Anti-Clumping Agent</td>
<td>20 mL</td>
<td>0010057AE</td>
</tr>
</tbody>
</table>
Introduction

Overview

Introduction

The CHO CD EfficientFeed™ Kit provides two supplements and a nutrient supplementation strategy designed to optimize cell growth and/or productivity in CHO cells. The two feed supplements, A and B, are based on the successful chemically defined media formulations, CD OptiCHO™ Medium or CD CHO Medium. Built using chemically defined components in higher concentrations and based on typical CHO cell culture requirements, these feeds demonstrate the capability for increasing cell growth and protein production by as much as 5-6 fold versus batch culture conditions. By using the two feed supplements independently or in combination, you can optimize fed-batch protocols for both cell growth and protein yield.

Nutrient Supplementation

Nutrient supplementation provides an extra metabolic “boost” to a cell culture so that growth and/or protein production continues to increase beyond what it would be with the inoculated cell culture medium alone. Cells will generally expand in a culture until nutrients are depleted and waste products increase to toxic levels. Numerous studies demonstrate the benefit of supplementing a culture with nutrients that are becoming depleted (see References, page 14). However, supplementation requires that only depleting components are in the supplement and not extra salts or non-depleting amino acids since osmolarity levels may become too high during supplementation. In addition, sufficient supplementation of key nutrients protects cells from damage induced by free radicals and apoptosis. The CHO CD EfficientFeed™ supplements provide only nutrients that generally become depleted in CHO cultures.

Criteria for Supplementation

Specific CHO cell lines and clones react differently to alternative supplementation protocols. Metabolic needs vary and it is essential for optimal growth and/or productivity to determine optimal nutrient supplementation protocols for each cell line or clone. You may find that two different feeds might be needed—one to optimize growth during scale-up and one to optimize protein production once scale-up is completed. Consider several general criteria to design supplementation protocol experiments:

- Although somewhat counter-intuitive, large supplementation (i.e. Day 0 supplementation) at culture initiation may provide superior cell growth and protein production yield for some CHO cell lines and clones.
- Nutrient supplementation feed levels above ~40-50% for CHO CD EfficientFeed™ supplements may negatively impact growth and productivity because of osmolarity and excess nutrient levels.
- Addition of supplements continuously over many days may additively increase osmolarity to detrimental levels.
- Combinations of Supplement A and B in addition to each alone may be tested to determine the optimal nutrient feed protocol.
Protocol Development Process

Introduction
The flow chart below outlines the overall process to determine the optimal combination of base medium and supplements to maximize cell growth and productivity.

Flow Chart
Methods

Compatibility Test

Introduction
In general when adding nutrient supplements to a medium it is important to know that the combination is stable. Potential concerns would include precipitation, chemical interaction, high osmolarity, and pH excesses. Compatibility testing is the process of determining the extent of incompatibility of each EfficientFeed™ supplement on the base medium (and other supplements).

Using GIBCO® Base Media
If using CD-OptiCHO™ or CD-CHO as the base medium with CHO CD EfficientFeed™ supplements, no compatibility testing is required since these media are compatible. We suggest growing cells in CD-OptiCHO™ or CD-CHO for three passages before feeding with CHO CD EfficientFeed™ supplements. Proceed to page 5 to optimize protocol.

Other Base Media or Supplements
If other base medium or additional supplements are used, perform the following compatibility test to ensure successful optimization of your protocol.

Reagents and Equipment Required
8 clear, sterile bottles that will hold 140 mL
1 liter of Base medium to test plus any additional supplements used
CHO CD EfficientFeed™ A
CHO CD EfficientFeed™ B
pH meter
Osmolarity meter

Preparation
Label the 8 bottles as follows: A (10%), A (20%), A (30%), A (40%), B (10%), B (20%), B (30%), and B (40%)

Continued on next page
Compatibility Test, continued

**Procedure**

1. Aseptically place 100 ml of base medium (plus any desired supplements) into the 8 bottles.
2. Add volumes of CHO CD EfficientFeed™ A or B based upon the 100 ml original volume. For example, add 10 ml (10%), 20 ml (20%), etc. of each supplement to the appropriate bottle.
3. Determine pH and osmolarity of each of the solutions to see whether any combinations fall outside of acceptable ranges.
   
   **Note:** In general, osmolarity should be below ~500 mOsm and pH should be ~6.5-8.0. However, some cell lines may be more resistant to changes in osmolarity and pH, so consult historical data for your particular cell line. In addition, increased osmolarity is known to stimulate protein production, so higher percentages of supplements should not necessarily be eliminated.
4. Cap the bottles and place at 37˚C for 24 hours.
5. Observe each supplementation level for precipitation and/or significant color change (will vary for different media) which could indicate component chemical reaction. Use your knowledge of your base medium and other supplements to evaluate the color changes. Those percentages that show no aberration are candidates for use in nutrient supplementation.
6. If all supplementation levels of one or both of the supplements indicate compatibility issues, contact Technical Service to assist resolution (page 13).

**PD-Direct™ Formulation Assistance**

To resolve more complex compatibility issues, you may wish to consult with our PD-Direct™ Formulation Assistance group. Our Media ADVANCE™ process improves cell culture yields through custom medium design following a rational approach proven to achieve cell performance up to multi–gram per liter yields. For more information on this service, please email pd.direct@invitrogen.com.
Supplementation Options: Day 0 Supplementation

Introduction
The variables to consider when designing an optimized nutrient supplementation strategy are a) the EfficientFeed™ supplements and combinations themselves and b) timing and quantity of each supplement to add to the culture. We present here two strategies for supplementation:

1. Day 0 Supplementation where cultures are supplemented only at Day 0 (recommended when culture maintenance time is limited).
2. Multi-Day Supplementation where supplementation occurs over several days to optimize cell growth and/or protein production. See page 7 for more information.

Using a Day 0 Supplementation Protocol
One of the advantages of using the CHO CD EfficientFeed™ Kit is that identifying a nutrient feeding strategy that significantly boosts cell growth and protein production of CHO cell lines is possible with minimal experimental time. Day 0 supplementation with the two CHO feeds independently or in combination allow rapid identification of the feeding strategy for specific cell lines.

Important
In general, adding up to a total supplementation of 40-50% over the life of the culture can yield improved protein production. Beyond that, osmolarity increases may become detrimental.

Reagents and Equipment Required
At least nine 20-30 ml CHO shake flask cultures started from the same initial culture (seeded at $2-3 \times 10^6$/mL and grown for 3 passages). More shake flask cultures are required if you want replicates for each data point.

Shaker (125 rpm)
CHO CD EfficientFeed™ A
CHO CD EfficientFeed™ B
Cell counting equipment

Continued on next page
Supplementation Options: Day 0 Supplementation, continued

Example of Day 0 Supplementation DOE

Performing a derivative of a 2 factor 3 level DOE feasibility is a relatively easy process for identifying a single-time feed nutrient supplementation strategy at Day 0. In addition, assessing ratios other than those indicated below may yield increased bioproductivity as part of a more in-depth DOE.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Supplement A volume %</th>
<th>Supplement B volume %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30*</td>
<td>30*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0</td>
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<tr>
<td>5</td>
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<td>6</td>
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<td>7</td>
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<td>8</td>
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<td>15</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The osmolarity levels at a 30% supplementation of both Supplement A and Supplement B may be too high for certain cell lines.

Sample Data

Cells were grown in CD OptiCHO™ Medium for three passages prior to the study. Cultures were seeded in triplicate at $2 \times 10^5$ viable cells/ml in 50 mL working volume in 250 mL shake flask. Cultures were incubated at 37°C, 8% CO₂, and shaken at 125 rpm. Only Day 0 feeds with different supplement combinations were used as illustrated in the legends. No further supplementation was performed during culture. Viable cell densities determined using Coulter ViCELL. Amount of EPO was determined by ELISA.

Fed-batch culture using CD OptiCHO as base medium recombinant EPO DG44 CHO

![Fed-batch culture using CD OptiCHO as base medium recombinant EPO DG44 CHO](image-url)
Supplementation Options: Multi-Day Supplementation

**Introduction**

To fully optimize cell growth or protein production, try to feed CHO cell cultures over multiple days of culture. A chart is provided to test 2 basic supplementation protocols. Other feed strategies are possible.

**Important**

Do not start supplementation with too high (>30%) of a supplementation percentage as this results in excessive osmolarity levels by the end of culture. Depending on your cell line, adding up to a total supplementation of 40-50% over the life of the culture can improve protein production. Beyond that, osmolarity increases may become detrimental.

**General Outline**

The protocol on the next page tests supplementation on days 2, 4, 6, and 8 as well as days 3, 6, 9, and 12.

Each culture starts with an initial supplementation at 15% culture volume on the first day of the culture and continuing at 10% volume at each subsequent time point.

**Reagents and Equipment Required**

At least twelve 20-30 ml CHO shake flask cultures in 125 ml flasks from the same initial culture (seeded at 2-3 × 10^5/mL and grown for 3 passages). Additional flasks may be needed for replication.

- Shaker (125 rpm)
- CHO CD EfficientFeed™ A
- CHO CD EfficientFeed™ B
- Cell counting equipment

**Cell Growth versus Productivity**

While the ultimate goal of CHO cell culture is increased protein production where supplement addition is to the bioreactor, several scale-up stages specifically for cell growth are usually required to get to the bioproduction stage. In this case, growth assays may indicate a different supplement protocol and ratios or alternate timings to maximize for growth instead of protein production.

**Examples of Results**

For several Invitrogen CHO cell lines cultured in CD OptiCHO™, conditions 1 and 5 (see chart next page and figure on page 9) have provided superior bioproducitivity results. It is better to optimize a supplementation protocol specifically for your cell lines, but if time is lacking these protocols may provide a significant bioproducitivity boost without performing a supplementation feasibility experiment.

*Continued on next page*
<table>
<thead>
<tr>
<th>Condition</th>
<th>Feeds</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>15</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<tr>
<td>2</td>
<td>A</td>
<td>15</td>
<td>10</td>
<td>%</td>
<td>%</td>
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<td>%</td>
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<td>3</td>
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<tr>
<td>5</td>
<td>A+B</td>
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<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<tr>
<td>6</td>
<td>A+B</td>
<td>15</td>
<td>10</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<td>7</td>
<td>A</td>
<td>10</td>
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<td>%</td>
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<tr>
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<tr>
<td>11</td>
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<td>%</td>
<td>%</td>
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<td>%</td>
<td>%</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>12</td>
<td>A+B</td>
<td>10</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Note**
As individual cell lines and clones will respond differently to supplementation, the chart provides guidance for initial studies and should result in significant bioproductivity boost, but optimal results may require cell-specific modifications of these supplementation protocols.

*Continued on next page*
Supplementation Options: Multi-Day Supplementation, continued

Sample Data

Cells were grown in CD OptiCHO™ Medium for three passages prior to the study. Cultures were seeded in triplicate at $3 \times 10^5$ viable cells/ml in 50 mL working volume in 250 mL shake flask. Cultures were incubated at 37°C, 8% CO₂, and shaken at 125 rpm. Different fed-batch strategies were used as illustrated in the legends. No further supplementation was performed during culture. Viable cell densities determined using Coulter ViCELL. IgG level determined by protein affinity HPLC. The IgG CHO cell line (Clone #1 from Chromos Molecular Systems) was developed by Chromos using ACE technologies.

Fed-batch culture using CD OptiCHO as base medium
Recombinant IgG Clone #1

![Graph showing viable cell density and IgG level over time for different fed-batch strategies. The graph includes data points for CD OptiCHO™ batch, Fed with 30% A/B mixture on day 0, Fed with 30% A on day 0, Fed with 15% A on day 0 and 10% on day 3, CD OptiCHO™ batch IgG, Fed with 30% A/B mixture on day 0 IgG, Fed with 30% A on day 0 IgG, and Fed with 15% A on day 0 and 10% on day 3 IgG. The graph illustrates a 5-fold increase and a 2-fold increase in IgG level.]
# Feed Additives

## Introduction

If further optimization is desired, additional suggestions are provided below. These suggestions should be tested prior to scale-up.

## Additional Glucose or Glutamine Supplementation

Glucose and glutamine may rapidly deplete in cultures even with the previous supplementation protocols. In these instances, it may be advantageous to supplement with a concentrate of glucose and/or glutamine, either empirically determined or based on monitoring of culture medium.

## Additional Amino Acid Supplementation

Quantifying all amino acids and glucose to construct a supplement of those components that have become depleted is another option. This requires a balanced supplement of components that are supplied in proportion to the extent of depletion of each as shown by SCM analysis. PD-Direct™ develops cell line-specific custom-designed balanced nutrient supplements.

## Hydrolysates

In general, a stock hydrolysate solution of ~100 g/L is made and cultures supplemented at concentration of 1-10g/L to provide useful increases in protein expression. Because of potentially high osmolarity and unknown components that may cause chemical reaction or precipitation, it is advisable to test for compatibility using the feeding protocol previously determined. If there are no compatibility issues, then test a range of concentrations to optimize protein production.
# Troubleshooting

## Introduction

Use the following table to troubleshoot any issues that may occur when developing your feeding protocol.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
</table>
| Compatibility testing indicates a problem (medium cloudy, obvious color change, flakes of precipitate, osmolarity too high) | Certain components exceeding solubility limits                         | Retest/Use with reduced supplementation percentage  
Configure alternative supplement or modify base medium to resolve  
Contact Technical Service or PD-Direct™ to resolve                 |
| Osmolarity exceeding levels the cells are known to tolerate.           | Retest/Use with reduced supplementation percentage  
Configure alternative supplement or modify base medium to resolve  
Contact Technical Service or PD-Direct™ to resolve                 |
| Contamination                                                          | Initiate new cultures, identify source of contamination via microbial testing |
| pH of supplemented medium too high or low.                             | Check/adjust pH of base medium                                           |
| Cells grow slowly                                                      | Cells from lag phase culture or not adapted to current medium.          | Subculture three passages in base medium prior to testing, keeping cells in log phase        |
| Osmolarity levels too high                                             | Reduce supplementation percentage, feed fewer times                    |
| Medium foamy                                                           | Lower the shaker speed slightly till no foam forms  
In bioreactor application, reduce sparging rate, check venting system  
Use FoamAway™ Irradiated, Cat. No. 0060096BC                          |
| Flasks too small (low O₂ transfer rate).                               | Use flasks that are at least 2.5 times bigger than the culture volume   |

*Continued on next page*
### Troubleshooting, continued

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells grow slowly, continued</td>
<td>Cells past subpassage limit</td>
<td>Initiate new culture</td>
</tr>
<tr>
<td></td>
<td>Cell culture clumpy</td>
<td>Increase mixing parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use Anti-clumping Agent, Cat. No. 0010057AE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Keep cells in log phase during scale-up</td>
</tr>
<tr>
<td></td>
<td>Cell culture parameters not correct</td>
<td>If bioreactor, check pH, sparging rate, impeller speed, etc.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If flasks, check incubator parameters, shaker speed, tighten caps, etc.</td>
</tr>
<tr>
<td></td>
<td>Low protein production</td>
<td>Under supplementation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perform % supplementation titration culture</td>
</tr>
<tr>
<td></td>
<td>CHO CD EfficientFeed™ improperly stored</td>
<td>Keep at refrigerated temperatures protected from light</td>
</tr>
<tr>
<td></td>
<td>Reduced cell protein expression</td>
<td>Initiate new culture from frozen stock, keeping cells in log phase</td>
</tr>
<tr>
<td></td>
<td>High osmolarity because of over-supplementation</td>
<td>Re-check calculations for volume addition</td>
</tr>
<tr>
<td></td>
<td>Not sampling at point of peak protein production</td>
<td>Sample over several days until culture viability decreases</td>
</tr>
<tr>
<td></td>
<td>IgG aggregation not detected on assay</td>
<td>Use gel to monitor product expression</td>
</tr>
<tr>
<td></td>
<td>Rapid cell growth, low protein expression</td>
<td>Energy shunted for expansion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consider alternative feeding strategies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consider temperature and/or pH shift to optimize</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact Technical Service or PD-Direct™</td>
</tr>
<tr>
<td></td>
<td>Glycosylation change</td>
<td>Ammonia/lactate levels too high</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Consider alternative feeding strategies</td>
</tr>
</tbody>
</table>
Technical Service

Web Resources  Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical service contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us  For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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Carlsbad, CA 92008 USA  3-9-15, Kaigan  3 Fountain Drive
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