invitrogen™

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

User Manual

Table of Contents

Kit Contents and Storage

Shipping/Storage

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

Item	Cat. No.	Shipping	Storage
CHO CD EfficientFeed™ Kit	A10241-01	Ambient	See below
CHO CD EfficientFeed™ A	A10234-01	Ambient	2-8˚C, protected from light
CHO CD EfficientFeed™ B	A10240-01	Ambient	2-8˚C, protected from light

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.

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

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:

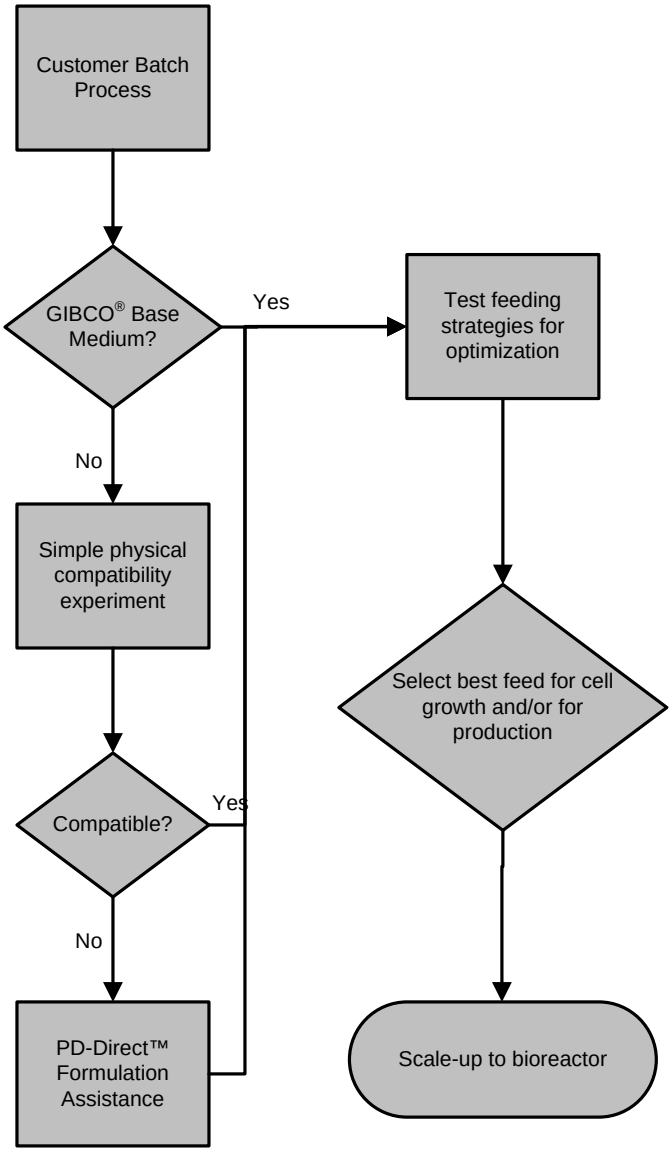
- Although somewhat counter-intuitive, large supplementation (i.e. Day 0 supplementation) at culture initiation may provide superior cell growth and protein production yield for some CHO cell lines and clones.
- Nutrient supplementation feed levels above ~40-50% for CHO CD EfficientFeed™ supplements 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 × 10^5/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

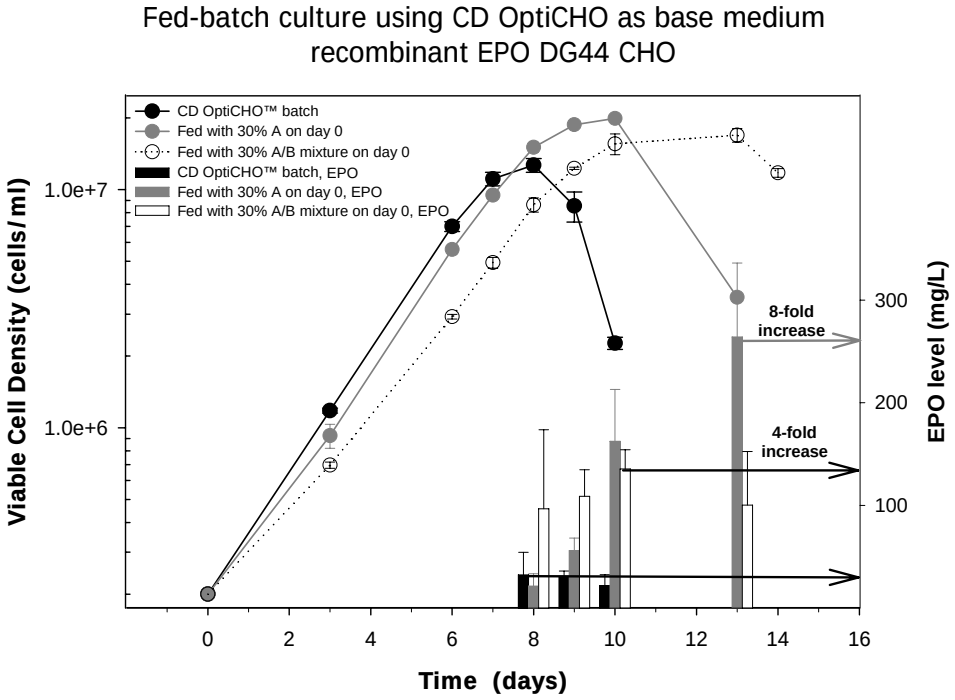
Performing a derivative of a 2 factor 3 level DOE feasibility is a relatively easy process for identifying a single-time feed nutrient supplementation strategy at Day 0. In addition, assessing ratios other than those indicated below may yield increased bioproductivity as part of a more in-depth DOE.

Condition	Supplement A volume %	Supplement B volume %
1	30*	30*
2	0	30
3	15	30
4	30	0
5	15	0
6	30	15
7	15	15
8	0	15
9	0	0

* 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 2×10^5 viable cells/ml in 50 mL working volume in 250 mL shake flask. Cultures were incubated at 37°C, 8% CO_2, 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.



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 bioproductivity 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 bioproductivity boost without performing a supplementation feasibility experiment.

Continued on next page

Supplementation Options: Multi-Day Supplementation, continued

Chart for Supplementation Testing

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Condition	Feeds	%		%	%	%		%		%	%			%
1	A	15			10			10			10			
2	A	15		10		10		10						
3	B	15			10			10			10			
4	B	15		10		10		10						
5	A+B	15			10			10			10			
6	A+B	15		10		10		10						
7	A			10		10		10		10				
8	A				10			10			10			10
9	B			10		10		10		10				
10	B				10			10			10			10
11	A+B			10		10		10		10				
12	A+B				10			10			10			10

Note

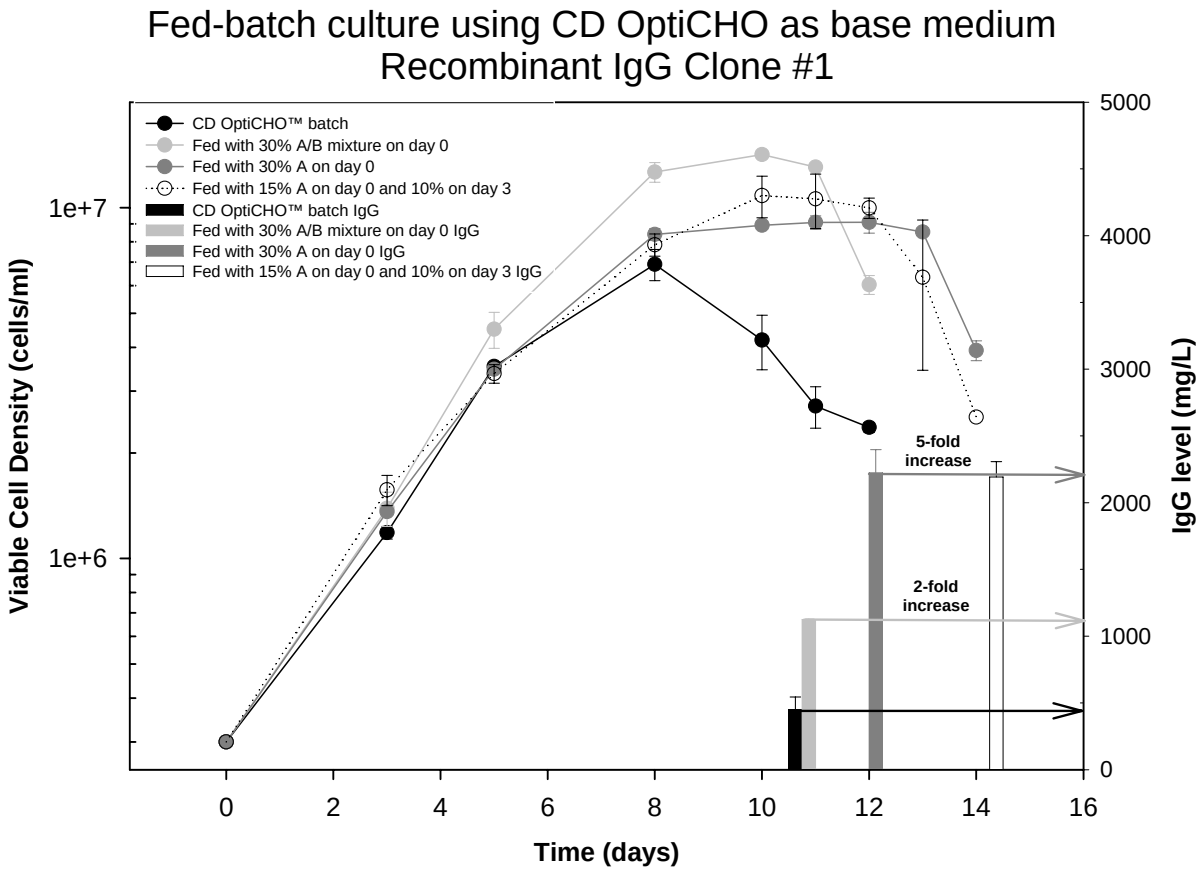
As individual cell lines and clones will respond differently to supplementation, the chart provides guidance for initial studies and should result in significant bioproductivity boost, but optimal results may require cell-specific modifications of these supplementation protocols.

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 x 10^5 viable cells/ml in 50 mL working volume in 250 mL shake flask. Cultures were incubated at 37°C, 8% CO_2, 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.



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.

Problem	Reason	Solution
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 O_2 transfer rate).	Use flasks that are at least 2.5 times bigger than the culture volume

Continued on next page

Troubleshooting, continued

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

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).

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
Invitrogen Japan
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Service Representatives.

Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

References

General Nutrient Supplementation
Birch, J. et al., (2006) "Antibody production" Adv. Drug Deliv. Rev.: 58(5-6), 671-85.

Burky, J., et al., (2007) "Protein-free fed-batch culture of non-GS NSO cell lines for production of recombinant antibodies" Biotechnol. Bioeng.: 96(2): 281-93.

Sandadi, S., et al., (2005) "Heuristic optimization of antibody production by Chinese hamster ovary cells" Biotechnol. Prog.: 21(5): 1537-42.

Apoptosis
Yeo, J. et al., (2006) "Glutamine or glucose starvation in hybridoma cultures induces death receptor and mitochondrial apoptotic pathways" Biotechnol. Lett.: 28(18): 1445-52.

Process Development
De Alwis, D., et al., (2007) "Statistical methods in media optimization for batch and fed-batch animal cell culture" Bioprocess Biosyst. Eng.: 30(2): 107-13.

Kuwae. S., et al., (2005) "Development of a fed-batch culture process for enhanced production of recombinant human antithrombin by Chinese hamster ovary cells" J. Biosci. Bioeng.: 100(5): 502-10.

Spens, E. et al., (2007) "Defined protein and animal component-free NSO fed-batch culture" Biotechnol. Bioeng.: (May 21: Epub).

Notes

Notes, continued

invitrogen™

Corporate Headquarters
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech.service@invitrogen.com

For country-specific contact information visit our web site at **www.invitrogen.com**