gibco

EfficientFeed™ + and GlycanTune™ Feeding Supplements Kits

USER GUIDE

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Contents

Product information

Product description

Feed Kit A+, B+, C +

Gibco™ Feed Kit A+, B+, C+ provides three supplements and a supplementation strategy designed to optimize cell growth and/or productivity in CHO cells. The three supplements, EfficientFeed™ A+ AGT™ Supplement, EfficientFeed™ B+ AGT™ Supplement, and EfficientFeed™ C+ AGT™ Supplement are based on the chemically defined CHO CD EfficientFeed™ A , CHO CD EfficientFeed™ B, and CD EfficientFeed™ C nutrient supplements. The EfficientFeed™+ AGT™ supplements are dry-format single-part supplements for fed batch addition to the culture of multiple cell lines to assist with process development and maximization of bioreactor utilization. Compared to original CHO CD EfficientFeed™ AGT™ nutrient supplements, EfficientFeed™+ supplements have components that are uniquely combined to allow simpler singlepart pH-neutral reconstitutions, as well as the ability to super-concentrate the supplements for reduction of product dilution or increased titers with additional feeding, when appropriate. By using the three feed supplements independently or in combination, you can optimize fed-batch protocols for both cell growth and protein yield.

Feed Kit A+, Feed Kit B+, and Feed Kit C+

Gibco™ Feed Kit A+, Feed Kit B+, and Feed Kit C+ each provide two chemically defined supplements and fed-batch supplementation strategies designed to optimize cell growth and protein production while offering the ability to target and fine tune glycosylation profiles. GlycanTune™ A+ Total Feed, GlycanTune™ B+ Total Feed, and GlycanTune™ C+ Total Feed are based on the chemically defined EfficientFeed™ A+, EfficientFeed™ B+ and EfficientFeed™ C+ supplements. The GlycanTune™ Total Feeds are feeding supplements specifically designed for a fed-batch culture of multiple cell lines. When used alone or with an EfficientFeed™+ supplement, this single-part total feeding supplement can shift glycan profiles from heavily G0F to mostly G1F and G2F glycans. The GlycanTune™ Total Feeds provides easy-to-reconstitute, pH-neutral solutions that can be super-concentrated for delivery of key nutrients at smaller volumes.

Contents and storage

- **Composition**: Chemically defined AGT™, protein free, plus supplements
- **Contains**: Carbon source, amino acids, vitamins, salts and trace minerals
- **Does not contain**: Lipids, hydrolysates, or growth factors

Contents are shipped on wet ice, store as described.

Table 1 Feed Kit A+, B+, C+ (Cat. No. A3315801)

Contents	Amount	Catalog Number	Storage
EfficientFeed™ A+ AGT™ Supplement	66.4 g/L (1L equivalent)	A25023	2°C to 8°C, protected from light
EfficientFeed™ B+ AGT™ Supplement	52.7 g/L (1L equivalent)	A25030	
EfficientFeed™ C+ AGT™ Supplement	81.2 g/L (1L equivalent)	A25031	
Flash drive with manual		—	Ambient

Table 2 Feed Kit A+ (Cat. No. A3315901)

Contents	Amount	Catalog Number	Storage
EfficientFeed™ A+ AGT™ Supplement	66.4 g/L (1L equivalent)	A25023	2°C to 8°C, protected from light
GlycanTune™ A+ Total Feed	73.0 g/L (1L equivalent)	A29719	
Flash drive with manual		—	Ambient

Table 3 Feed Kit B+ (Cat. No. A3316001)

Contents	Amount	Catalog Number	Storage
EfficientFeed™ B+ AGT™ Supplement	52.7 g/L (1L equivalent)	A25030	2°C to 8°C, protected from light
GlycanTune™ B+ Total Feed	59.2 g/L (1L equivalent)	A29720	
Flash drive with manual		—	Ambient

Table 4 Feed Kit C+ (Cat. No. A3316101)

Contents	Amount	Catalog Number	Storage
EfficientFeed™ C+ AGT™ Supplement	81.2 g/L (1L equivalent)	A25031	2°C to 8°C, protected from light
GlycanTune™ C+ Total Feed	87.7 g (1L equivalent)	A29721	
Flash drive with manual		—	Ambient

Protocol development process

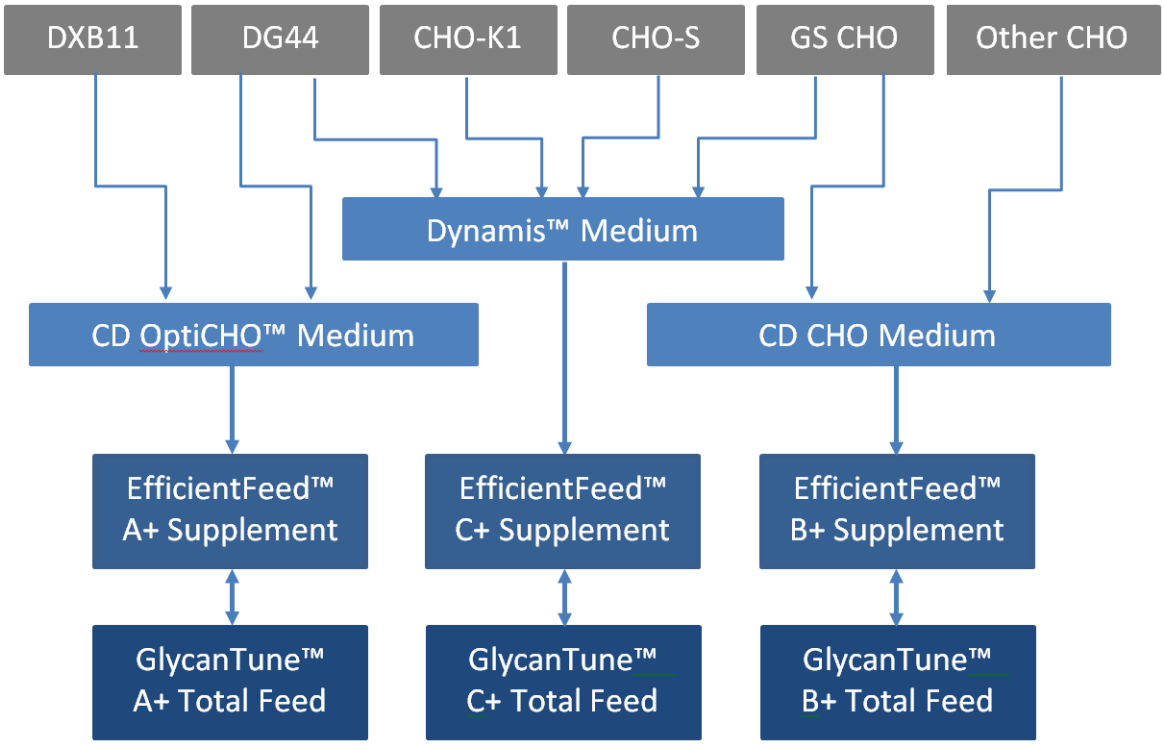

Figure 1 Overall process to determine the optimal combination of base medium and supplements to maximize cell growth and productivity

Reconstitution recommendations

The EfficientFeed™+ Supplements and the GlycanTune™+ Total Feeds are designed with the flexibility to super-concentrate chemically defined components up to 3X (i.e., 200 g/L) of original levels while remaining pH-neutral. This is accomplished with nutrient delivery technology that allows reconstitution without pH adjustments (i.e., just add water). By adding different amounts of water during reconstitution, you can concentrate these supplements to 2X, or up to 3X. This provides the ability to optimize bioreactor output by reducing product dilution, maximization of working volume and an increase of nutrients for increased titer.

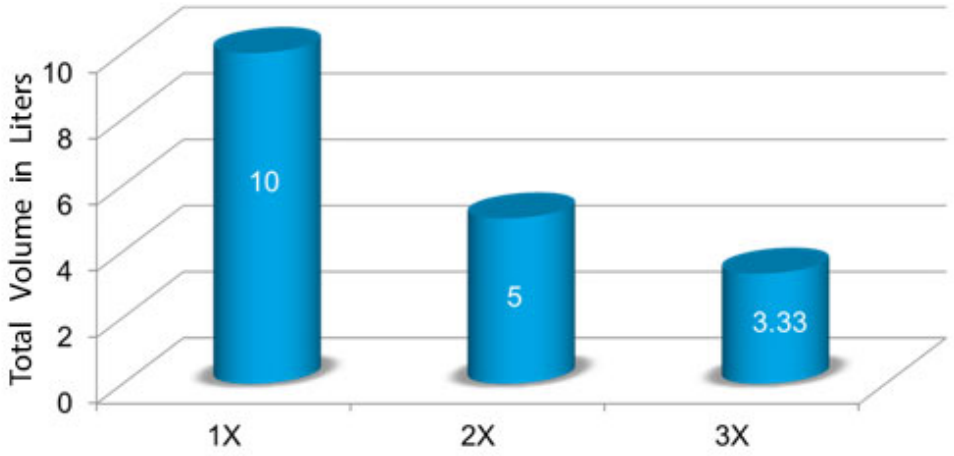

Figure 2 Comparison of final volumes for reconstituting a 10 L package of either an EfficientFeed™+ Supplement or GlycanTune™+ Total Feed

Super-concentrate the supplements

Weight out amount as indicated to make a 3X solution or 2X solution and dissolve as noted.

Product	Concentration	Dissolution time
EfficientFeed™ A+ AGT™ Supplement	1X (66.4 g/L)	20 min
	3X (199.2 g/L)	75 min
EfficientFeed™ B+ AGT™ Supplement	1X (52.7 g/L)	20 min
	3X (158.1 g/L)	50 min
EfficientFeed™ C+ AGT™ Supplement	1X (81.2 g/L)	40 min
	2X (162.4 g/L)	75 min
GlycanTune™ A+ Total Feed	1X (73 g/L)	20 min

Product	Concentration	Dissolution time
GlycanTune™ A+ Total Feed	3X (219.0 g/L)	75 min
GlycanTune™ B+ Total Feed	1X (59.2 g/L)	20 min
	3X (177.6 g/L)	50 min
GlycanTune™ C+ Total Feed	1X (87.7 g/L)	40 min
	2X (175.4 g/L)	75 min

Compatibility

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 excess. Compatibility testing is the process of determining the extent of incompatibility of each EfficientFeed™+ Supplement and GlycanTune™+ Total Feed on the base medium (and other supplements).

Gibco™ base medium

If using CD CHO Medium, CD OptiCHO™ Medium or Dynamis™ Medium as the base medium with either or both EfficientFeed™+ and GlycanTune™+ supplements, no compatibility testing is required since these media are compatible. We suggest growing cells in CD CHO Medium, CD OptiCHO™ Medium or Dynamis™ Medium for three passages before feeding with EfficientFeed™+ Supplements and/or GlycanTune™+ Total Feeds.

Perform compatibility testing

If other base medium or additional supplements are used, perform compatibility testing by simulating cell culture testing using the following procedure.

1. Aseptically place 100 mL of base medium (plus any desired supplements) into the bottles.
2. Add volumes of EfficientFeed™ (A+, B+, C+) and/or GlycanTune™ (A+, B+, C+) based upon the 100 mL original volume. For example, add 30 mL (30%), 40 mL (40%), 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.

Gibco™ Bioproduction Services assistance

To resolve more complex compatibility issues, you may wish to consult with our Gibco™ Bioproduction Services group. Our media optimization 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, see **thermofisher.com/gibcobpdservices**.

EfficientFeed™ + supplementation

The variables to consider when designing an optimized nutrient supplementation strategy are a) the EfficientFeed™ supplements and/or combinations themselves and b) timing and quantity of each supplement to add to the culture. It is recommended to use multi-day supplementation where supplementation occurs over several days to optimize cell growth and/or protein production.

Required materials

At least 24 x 30 mL CHO shake flask cultures in 125 mL-flasks from the same initial culture (seeded at $3x10^5$ cells/mL and grown for 3 passages). Additional flasks may be added for replication.

- Shaker (125rpm)
- EfficientFeed™ A+ AGT™ Supplement
- EfficientFeed™ B+ AGT™ Supplement
- EfficientFeed™ C+ AGT™ Supplement
- Cell counting equipment

Supplementation options

			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Condition	Feed	Concentration	%	%	%	%	%	%	%	%	%	%	%	%
1	A+	1X					10		10		10		10	
2	A+	3X					3.3		3.3		3.3		3.3	
3	A+	3X					1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
4	B+	1X					10		10		10		10	
5	B+	3X					3.3		3.3		3.3		3.3	
6	B+	3X					1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
7	C+	1X					10		10		10			
8	C+	2X					5		5		5			
9	C+	2X					1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9

Note: Individual cell lines and clones 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.

EfficientFeed™ + Supplement sample conditions

Cells were grown in either CD OptiCHO™ Medium, CD CHO Medium or CD FortiCHO™ Medium for at least three passages prior to the study. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 55 mL working volume in 250 mL shake flasks. Cultures were incubated at 37°C, 8% CO_2, and shaken at 125 rpm. Glucose was fed at 3 g/L when measured glucose was at or below 3 g/L. Different fed-batch strategies were used as illustrated in the legends. No further supplementation other than indicated was performed during culture. IgG concentrations measured using a Roche Cedex Bio HT.

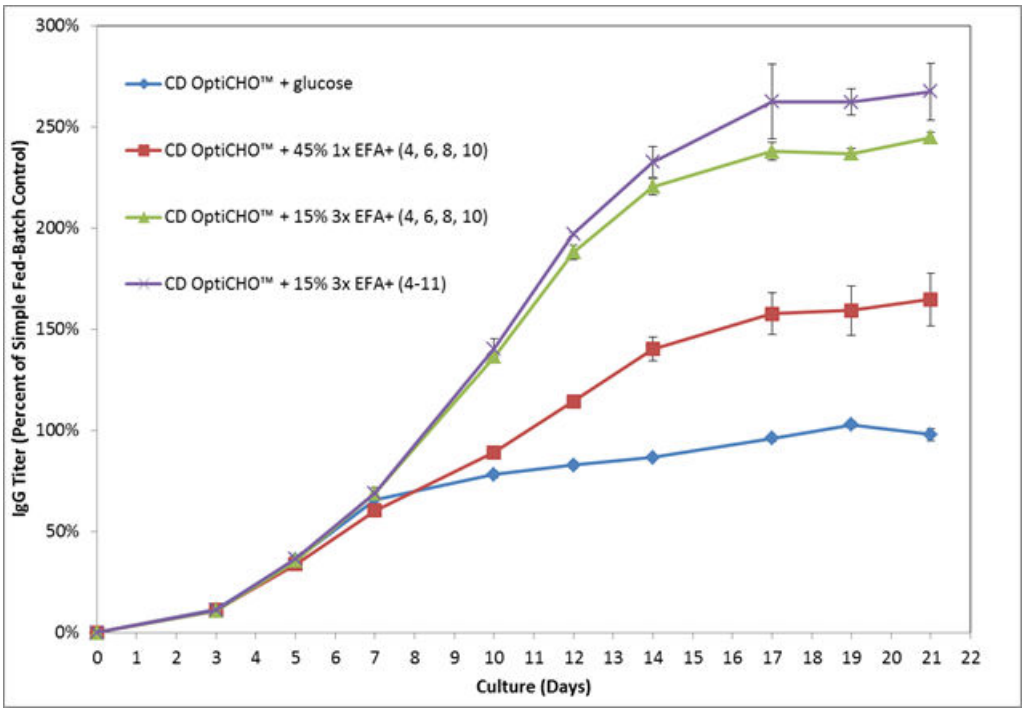

Figure 3 EfficientFeed™ A+ AGT™ Supplement sample IgG data

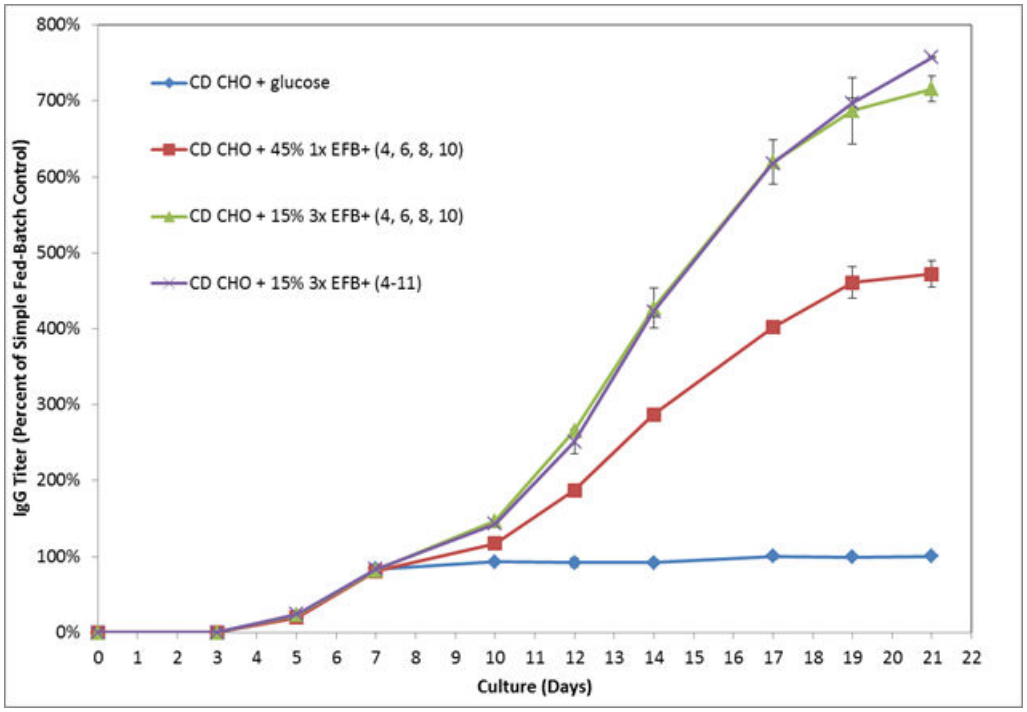

Figure 4 EfficientFeed™ B+ AGT™ Supplement

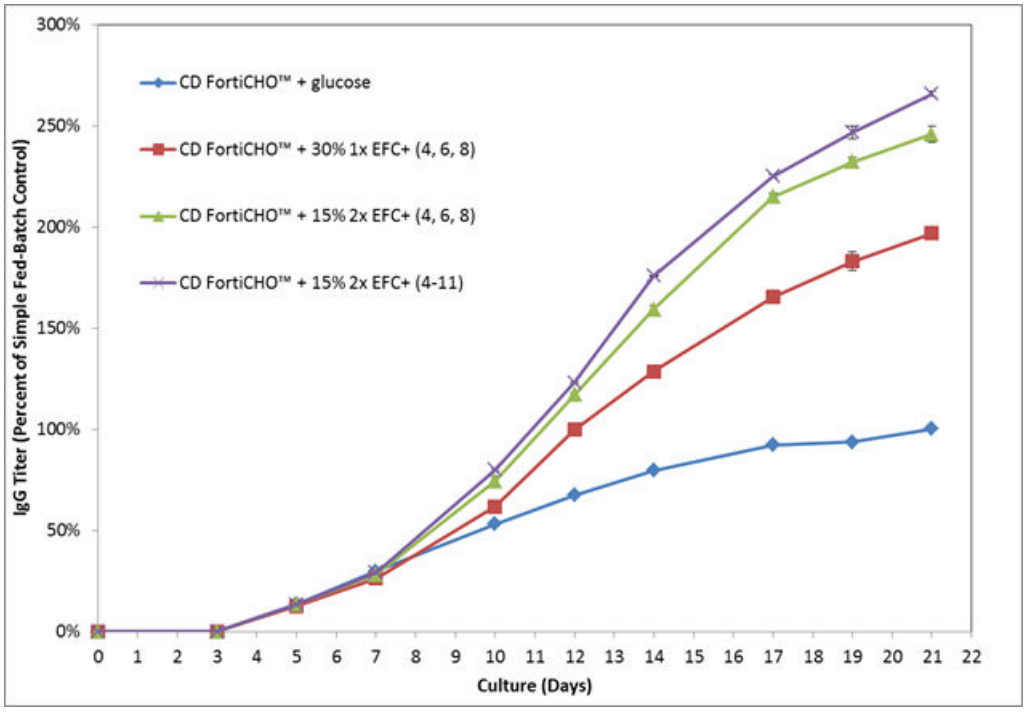

Figure 5 EfficientFeed™ C+ AGT™ Supplement

GlycanTune™ + Total Feed supplementation

GlycanTune™ A+, B+ or C+ Total Feed can be added as a standalone process that can result in a significant shift from G0F to G1F and G2F (maximum galactosylation). Using a unique fed-batch process, the feed can also be used with an original process feed such as EfficientFeed™ A+, B+ or C+ Supplements to dial in a targeted glycosylation profile. This can be achieved through a process where a transition point is used to switch from the EfficientFeed™ A+ AGT™ Supplement to the GlycanTune™+ Total Feed. The timing of the transition point will determine the specificity of the glycan profile. A transition point early in culture will result in a greater shift from G0F to G1F and G2F. A transition midway or late in culture will result in a greater proportion of G0F compared to G1F and G2F.

Note: As individual cell lines and clones will respond differently to supplementation, the charts below provide guidance for initial studies and should result in shifts from G0F to G1F and G2F, but optimal results may require cell-specific modifications of these supplementation protocols.

Require materials

At least 16 x 30 mL CHO shake flask cultures in 125 mL flasks from the same initial culture (seeded at $3x10^5$ cells/mL and grown for 3 passages). Additional flasks may be added for replication.

- Shaker (125 rpm)
- EfficientFeed™ A+ AGT™ Supplement (EFA+) and/or GlycanTune™ A+ Total Feed (GTA+)
- EfficientFeed™ B+ AGT™ Supplement (EFB+) and/or GlycanTune™ B+ Total Feed (GTB+)
- EfficientFeed™ C+ AGT™ Supplement and/or GlycanTune™ C+ Total Feed (GTC+)
- Cell counting equipment
- Method and instumentation to analyze protein glycosylation

GlycanTune™ A+ supplementation options

			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	Day 13	Day 14	Day 15
Condition	Feed 1	Feed 2	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	3X EFA+						1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
2	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
4	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
5	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
6	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
7	3X EFA+	3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
8		3X GTA+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

GlycanTune™ A+ Total Feed Supplement sample data

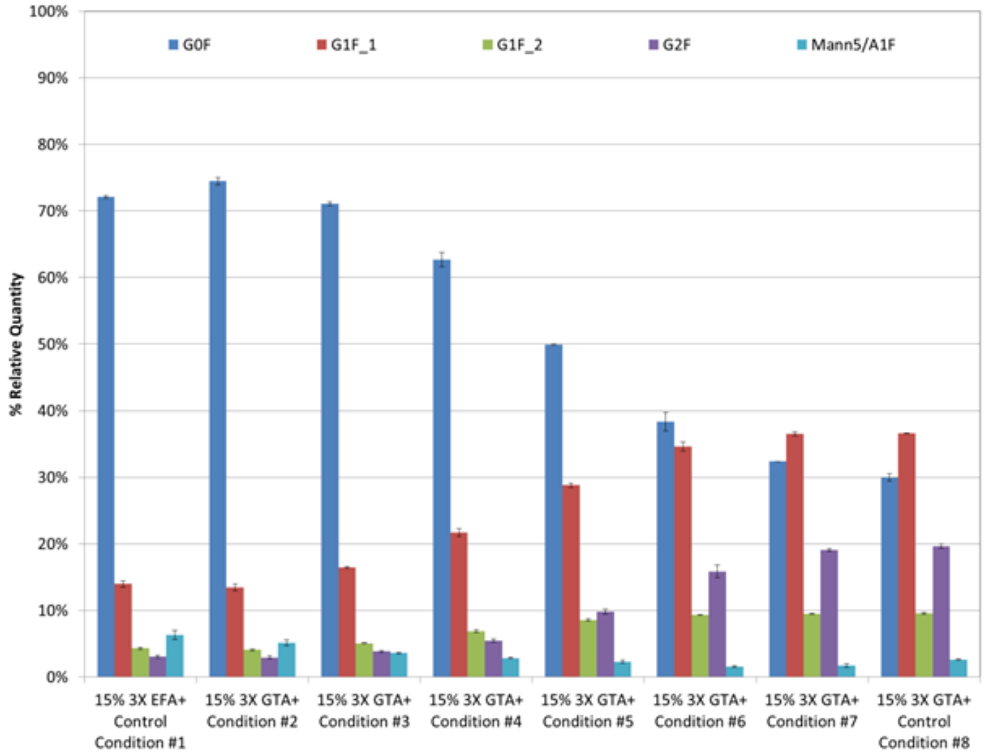

Figure 6 Glycosylation analysis results

Cells were grown in CD OptiCHO™ Medium for at least three passages prior to the study. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 60 mL working volume in 250 mL shake flasks. Cultures were incubated at 37°C, 8% CO_2, and shaken at 125 rpm. Glucose was fed at 3 g/L when measured glucose was at or below 3 g/L. Different fed-batch strategies were used. No further supplementation other than indicated was performed during culture. IgG concentrations measured using a Roche Cedex Bio HT. Glycosylation profiles were analyzed using a PNGaseF digestion with APTS labeling followed by detection on an ABI 3500 Genetic Analyzer.

GlycanTune™ B+ supplementation options

			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	Day 13	Day 14	Day 15
Condition	Feed 1	Feed 2	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	3X EFB+						1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
2	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
4	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
5	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
6	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
7	3X EFB+	3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
8		3X GTB+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

GlycanTune™ B+ Total Feed Supplement sample data

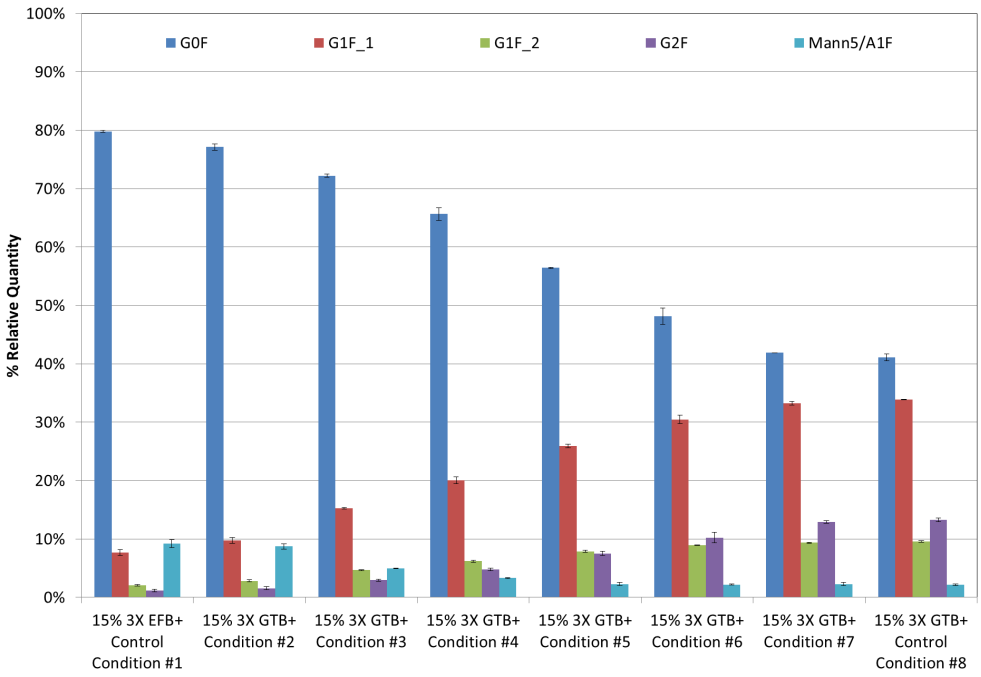

Figure 7 Glycosylation analysis results

Cells were grown in CD CHO Medium for at least three passages prior to the study. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 60 mL working volume in 250 mL shake flasks. Cultures were incubated at 37°C, 8% CO_2, and shaken at 125 rpm. Glucose was fed at 3 g/L when measured glucose was at or below 3 g/L. Different fed-batch strategies were used. No further supplementation other than indicated was performed during culture. IgG concentrations measured using a Roche Cedex Bio HT and glycosylation profiles were analyzed using a PNGaseF digestion with APTS labeling followed by detection on an ABI 3500 Genetic Analyzer.

GlycanTune™ C+ supplementation options

			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	Day 13	Day 14	Day 15
Condition	Feed 1	Feed 2	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	2X EFC+						1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
2	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
4	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
5	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
6	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
7	2X EFC+	2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
8		2X GTC+					1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25

GlycanTune™ C+ Total Feed sample data

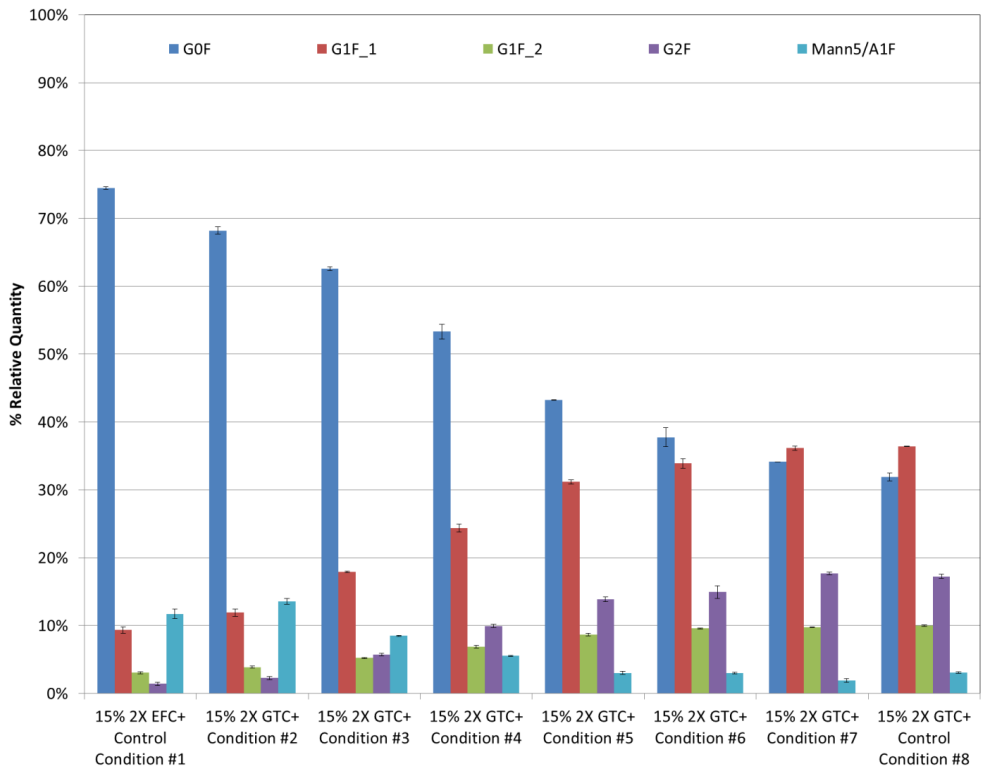

Figure 8 Glycosylaion analysis results

Cells were grown in Dynamis™ Medium for at least three passages prior to the study. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 60 mL working volume in 250 mL shake flasks. Cultures were incubated at 37°C, 8% CO_2, and shaken at 125 rpm. Glucose was fed at 3g/L when measured glucose was at or below 3g/L. Different fed-batch strategies were used. No further supplementation other than indicated was performed during culture. IgG concentrations measured using a Roche Cedex Bio HT and Glycosylation profiles were analyzed using a PNGaseF digestion with APTS labeling followed by detection on an ABI 3500 Genetic Analyzer.

GlycanTune™ + Supplement options with other media and feed supplements

GlycanTune™ C+ Total Feed Supplement sample data with EMD Millipore Cellvento™ CHO-200 Medium and Cellvento™ Feed-200

			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	Day 13	Day 14
Condition	Feed 1	Feed 2	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	Cellvento™ Feed-200						3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
2	Cellvento™ Feed-200	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
3	Cellvento™ Feed-200	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
4	Cellvento™ Feed-200	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
5		1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6

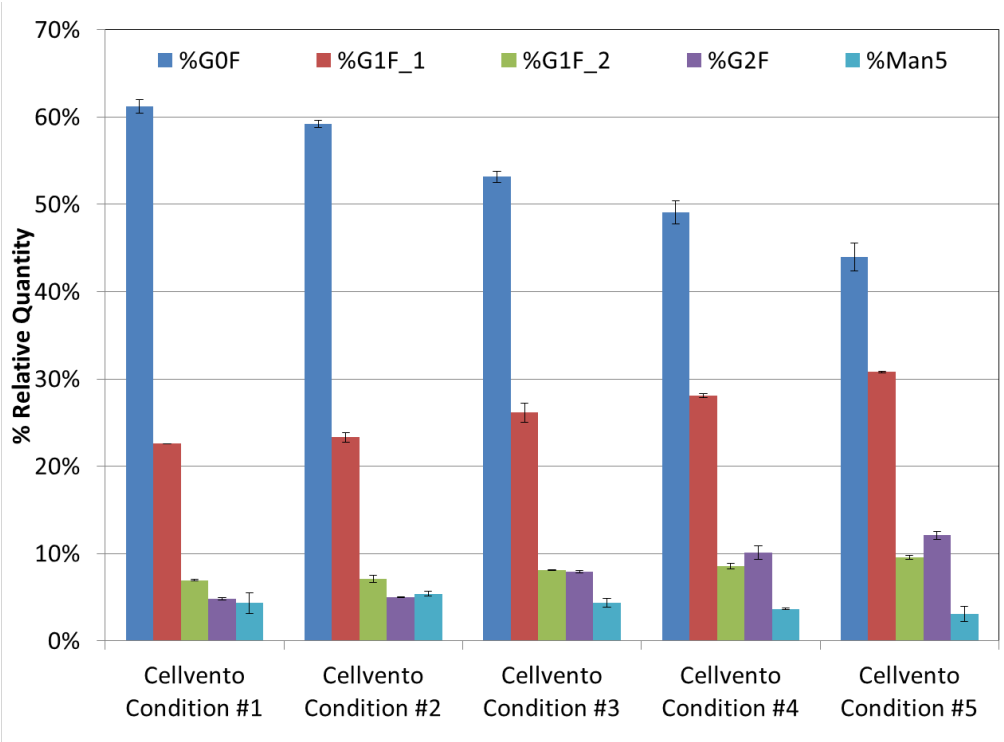

Figure 9 Glycosylation analysis results

Cells were grown in Cellvento™ CHO-200 Medium for at least three passages prior to the study to adapt cells. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 13mL working volume in 15 mL Ambr 15 micro bioreactors. The ambr 15 parameter we set at 37°C, 50% DO, and a mixer speed of 800 rpm. Glucose was fed at 3 g/L when measured glucose was at or below 3 g/L. Different fed-batch strategies transitioning from Cellvento™ Feed-200 to GlycanTune™ C+ were used as illustrated. No further supplementation other than indicated was performed during culture. Glycosylation profiles were analyzed using a PNGaseF digestion with APTS labeling followed by detection on an ABI 3500 Genetic Analyzer.

GlycanTune™ C+ Total Feed Supplement sample data with SAFC EX-CELL Advanced CHO Fed-batch Medium and EX-CELL Advanced CHO Fed-batch Feed 1

			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	Day 13	Day 14
Condition	Feed 1	Feed 2	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
1	SAFC Feed 1						3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
2	SAFC Feed 1	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
3	SAFC Feed 1	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
4	SAFC Feed 1	1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
5		1X GTC+					3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6

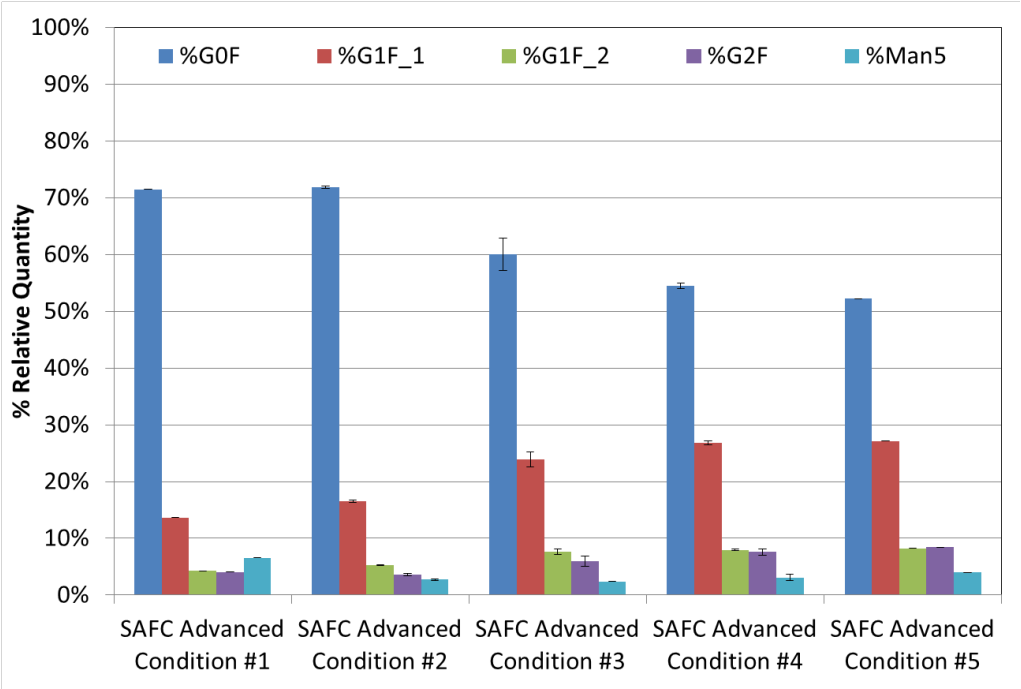

Figure 10 Glycolation analysis results

Cells were grown in SAFC EX-CELL Advanced CHO Medium for at least three passages prior to the study to adapt cells. Cultures were seeded in duplicate at 3 x 10^5 viable cells/mL in 13 mL working volume in 15 mL Ambr 15 micro bioreactors. The ambr 15 parameter we set at 37°C, 50% DO, and a mixer speed of 800 rpm. Glucose was fed at 3 g/L when measured glucose was at or below 3 g/L. Different fed-batch strategies transitioning from SAFC EX-CELL Advanced CHO Feed 1 to GlycanTune™ C+ were used as illustrated. No further supplementation other than indicated was performed during culture. Glycosylation profiles were analyzed using a PNGaseF digestion with APTS labeling followed by detection on an ABI 3500 Genetic Analyzer.

Feed additives

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. Gibco™ Bioproduction services can develop cell line-specific custom-designed balanced nutrient supplements.

FunctionMAX™ TiterEnhancer

There may be instances where specific productivity decreases late in a high density fed-batch culture. For these situations, FunctionMAX™ TiterEnhancer was designed to reinforce existing feeds and amplify the productivity of standard high cell density fed-batch platforms. FunctionMAX™ TiterEnhancer can be added on top of existing processes or as a partial feed replacement which can help to maintain specific productivity throughout culture up until harvest.

Hydrolysates

In general, a stock hydrolysate solution of ~100 g/L is made and cultures supplemented at concentration of 1–10 g/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.

A Troubleshooting

Observation	Possible cause	Recommended action
Compatibility testing indicates a problem (medium cloudy, obvious color change, flakes of precipitate, osmolarity too high)	Certain components exceed solubility limits	Retest/use with reduced supplementation percentage
		Configure alternative supplement or modify base medium to resolve
		Contact Gibco™ Bioproduction Services 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 Gibco™ Bioproduction Services to resolve
	Contamination	Initiate new cultures, identify source of contamination via microbial testing
	The pH of supplemented medium is 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
		UseFoamAway™ Irradiated FoamAway™ (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
	Cells past subpassage limit	Initiate new culture
	Cells culture clumpy	Increase mixing parameters
		Use Anti-clumping Agent (Cat. No. 0010057AE) at an initial concentration of 1 to 1000 dilution
		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.

Observation	Possible cause	Recommended action
Low protein production	Under supplementation	Perform % supplementation titration culture
	EfficientFeed™+ Supplement and/or GlycanTune™+ Total Feed 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 Gibco™ Bioproduction Services to resolve
Glycosylation change	Ammonia/lactate levels too high	Consider alternative feeding strategies
	Percentage of G1F and G2F too low	Consider feeding strategies using GlycanTune™ + Total Feed
		Transition to GlycanTune™+ Total Feed at an earlier time point
		Contact Gibco™ Bioproduction Services to resolve
	Percentage of G1F and G2F too high	Consider feeding strategies either not using or using less GlycanTune™+ Total Feed
		Transition to GlycanTune™+ Total Feed at a later time point
		Contact Gibco™ Bioproduction Services to resolve

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier. For more information see**http://www.thermofisher.com/us/en/home/life-science/bioproduction.html** or contact Technical Support.

Item	Amount	Source
CD OptiCHO™ Medium, (1X) liquid	1000 mL	12681
CD CHO Medium, (1X) liquid	1000 mL	10743
Dynamis™ Medium, (1x) liquid	1000 mL	A26615
EfficientFeed™ A+ AGT™ Supplement	66.4 g/L (1 L equivalent)	A25023
EfficientFeed™ B+ AGT™ Supplement	52.7 g/L (1 L equivalent)	A25030
EfficientFeed™ C+ AGT™ Supplement	81.2 g/L (1 L equivalent)	A25031
GlycanTune™ A+ Total Feed	73.0 g/L (1 L equivalent)	A29719
GlycanTune™ B+ Total Feed	59.2 g/L (1 L equivalent)	A29720
GlycanTune™ C+ Total Feed	87.7 g/L (1 L equivalent)	A29721
GlycanAssure™ APTS Kit	—	A28676
GlycanAssure™ Teal™ Kit	—	A28677
GlycanAssure™Turquoise™ Kit	—	A28678
GlutaMAX™ Supplement	100 mL	35050-061
Anti-clumping Agent	20 mL	0010057AE
Water, Distilled	—	15230
3500 Genetic Analyzer for Protein Quality	—	A30467

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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